Nutrient Sources for Organic Production: Soil Biological and Agronomic Responses

by

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II. Authors’ Contributions

L.V.M. performed the experiments, collected, and analyzed the data. M.H.E. conceived the original idea, helped to interpret the data, revised, and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

III. DEDICATION

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VII. ABSTRACT

Mukungu, Laetitia V. M.Sc., University of Manitoba, 2024. Nutrient sources for organic production: soil biological and agronomic responses. Major Advisor: Martin H. Entz.

Organic sources of phosphorus tend to be limited, expensive, or in danger of depletion. This has led phosphorus deficiency to become a common challenge in many Canadian organic farms. This study was divided into two chapters that evaluated various organic soil amendments as alternative sources of Phosphorus (P) under field and greenhouse conditions. The first chapter was a field study that evaluated the effect of a long-term P mitigation strategy on P sufficiency and arbuscular mycorrhizal fungal (AMF) infection in flax (*Linum usitatissimum*) grown in a long-term field study in Manitoba. The two main objectives of the study were to test if manure addition improved plant performance in terms of P tissue concentration, AMF, and biomass and whether the changes in AMF were related to plant's P uptake. Flax was planted after wheat (*Triticum aestivum*) in a 4-year – alfalfa (*Medicago sativa*) – alfalfa – wheat rotation under three treatments: organic manured (ORG M), organic non-manured (ORG NM), and conventional (CONV). The manure blend (80 kg/ha P) applied was a mixture of black soldier fly frass, anaerobic digestate of food waste, and beef cattle compost (1/3 of each) added once per rotation cycle in the fall of the first alfalfa year. Flax was sampled at flowering phase; after 52 days of growth and assessed for above-ground biomass, P tissue concentration, plant P uptake, and AMF root colonization. The benefits of manure addition on flax growth were observed in a wet year (685 mm annual precipitation) compared with a drier year (321mm), providing evidence that soil moisture is required to facilitate P movement within the soil. Under wet soil conditions, the addition of manure to the organic plots increased biomass by 33%; ORG M treatment produced higher biomass than ORG NM. Manure did not have a significant effect on flax P tissue concentration under both dry and wet soil conditions; adding manure did not improve flax P sufficiency as the ORG NM treatment presented 8% more P tissue concentration than the ORG M. AMF abundance was not significantly different in organic manured and non-manured treatment under wet soil conditions. However, under dry soil conditions, AMF colonization was 22% higher in non-manured than manured. A decreased abundance of AMF was observed when P fertilizer was added to the soil especially under wet conditions. However, flax amended with manure had higher AMF

colonization than MAP-amended treatment. An increase in AMF led to a slight increase in P tissue concentration under the organic conditions.

The second chapter investigated various organic soil amendments as alternative sources of P under greenhouse conditions. The general aim of the study was to test the performance of different organic soil amendments from various sources including livestock, insects, and food waste to expose farmers to more diverse options for their organic soil nutrients. The experiment had three objectives: (1) Assess if there were treatment differences among the organic amendments in terms of biomass, P uptake, and AMF colonization; (2) Evaluate rabbit manure as a novel manure source and compare its performance when in natural pellet from versus ground form; and (3) Investigate if AMF colonization affects the ability of soybeans to uptake P. Soybean was planted in P-depleted soil (3ppm Olsen-P) amended with four organic nutrients: composted beef cattle manure (CM), anaerobic digestate (AD), black soldier fly frass (FR), rabbit manure droppings (RM), ground rabbit manure (GRM) and compared to Mono-Ammonium Phosphate (MAP) and a control (CONT) (no amendments). The amendments were all added at a rate equivalent to 20 kg P ha^{-1} and the amendments per pot were 13.1 g CM, 1.5 g AD, 4.6 g FR, 13 g RM, 0.18 g MAP, and 13 g GRM. Soybean was sampled for above-ground biomass, P tissue concentration, plant P uptake, and AMF colonization at the initial phase of flowering, 42 days after planting. GRM treatment produced the highest biomass, 34% higher ($P<0.05$) than RM, 35% more than CONT, and 19% more than the mineral fertilizer, MAP. Similarly, GRM had the highest P tissue concentration, 18% higher than RM, 23% higher than CONT, and 9% more than MAP. Some of the organic amendments including GRM and frass were observed to be very competitive against their organic counterparts and even mineral fertilizer, MAP.

1. GENERAL INTRODUCTION

The majority of food producers in the Canadian prairies have maintained soil productivity by applying chemical agricultural inputs such as fertilizers to maintain crop yields (Walburger et al., 2004). The adoption of these fertilizers tends to be rapid and wide because they are largely evaluated solely based on their economic efficiencies in production such as the quick increased yield production and lower production cost while little attention is given to their potential environmental hazards (Zhang et al., 2018). However, the long-term heavy use of chemical fertilizers has resulted in alarming negative consequences on the environment and its people resulting in problematic outcomes such as eutrophication of waters, soil acidification, nitrate leaching, and loss of biodiversity (Chandini et al., 2019). Further research suggests that the massive use of chemical fertilizers world-wide is associated with the accumulation of heavy metals such as arsenic, cadmium, lead, and mercury in agricultural soils (Zhang et al., 2018). Additionally, fears about pesticide residues in conventionally produced foods heightened concerns about food safety (Lockeretz, 2007), hence the advocacy for a return to environmentally-friendly food production systems. As a result, producers in some parts of the Canadian Prairies have adopted more environmentally sustainable farming practices such as organic farming. In Canada, the prairies represent 50% of all organic land used for agriculture (Carkner et al., 2023).

Organic farming relies on biological nutrient cycling and practices such as green manure plow downs and legume N-fixation, which reduce the risk of aquatic pollution (Hansen et al., 2001). Organic agriculture encompasses a comprehensive approach that incorporates various sustainable farming principles such as farm biodiversity, minimal/no tillage, crop rotation, maintaining permanent soil cover, utilizing animal manure, green manure, and legumes (Meena, 2013). IFOAM (2008) outlined four fundamental principles of organic agriculture: Health, Ecology, Fairness, and Care, which dictated their definition of organic farming as, "A production system that sustains the health of soils, ecosystems, and people. It relies on ecological processes, biodiversity, and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation, and science to benefit the shared environment."

The adoption of organic farming in the Canadian Prairies faces some challenges: First, there is uncertainty from farmers that this alternative solution could be environmentally friendly but not economically viable and sustainable for them. Second, the concept of organic farming is poorly understood by many farmers and to date, extension education on organic farming is limited (Walburger et al., 2004). Third, nutrient management, especially P, is a major challenge for many organic farmers facing P deficiency (Entz et al., 2001; Welsh et al., 2009). According to Reid et al. (2019), the main drivers of P deficiency in Canada include (1) Lack of a consistent, P-replacing system on farms where P is removed in the harvested portion of the crops such as grains; (2) Location of some farms in remote areas far from fertilizer supply raising transportation and application costs of P fertilizers; and (3) Strong rental competition especially for cropland under the rent cash system; the competition creates little assurance about the availability of the land for multiple cropping seasons, and hence, no interest to invest in crop inputs like P fertilizers beyond the leased period. Four, limited availability of acceptable organic soil amendments frustrates farmers' efforts to implement organic farming. Currently, the approved P fertilizer options for organic use are manure and rock phosphate (Carkner et al., 2023). Unlike N which can be fixed through inclusion of legumes, green manures, and forages like alfalfa (*Medicago sativa*) within the crop rotations, P can only be added through approved organic amendments which, most times, are scarce. Rock phosphate presents low solubility especially when applied in high pH soils, a common characteristic of most organic Canadian soils, thus its nutrient provision to plants is limited mostly in the first year of application (Martin et al., 2007; Arcand and Schneider, 2006). Manure challenges vary depending on the farm's situation. Some farmers, especially those owning large acreage farms that require large amounts of manure, find manure to be very expensive to purchase and transport. Moreover, very few organic farms in the prairies host livestock (Carkner et al., 2023; Knight et al., 2010) and even those that do still face insufficiency as the farm size requires more manure than is available. Although currently the National Organic Program standards approve manure from conventional farming operations to be applied on organic farms, there continues to be doubts based on the claim that the composting process does not completely destroy modified transgenic DNA (Martin et al., 2007a). Furthermore, some conventionally-produced manures may be contaminated with pesticides, heavy metals, hormones, and antibiotics residues.

Based on these limitations, there have been efforts to explore other potential organic sources of P to give farmers more options. Some of the unconventional sources currently being explored on organic farms and that are part of this study include frass from black soldier fly larvae and anaerobic digestate from food waste (Nicksy and Entz, 2021). The widespread adoption of organic agriculture prompted extensive research efforts aimed at disseminating knowledge and providing guidance to farmers. Educational strategies included public outreach and farmer-to-farmer exchange programs.

The present study has three research chapters. The first chapter is the literature review of ‘Nutrient sources for organic production: soil biological and agronomic responses.’ This chapter covers both the early sources of organic nutrients such as wood ash, bird guano, fish waste, and the alternative emerging sources such as insect frass, anaerobic digestate, and rabbit manure. This literature review also includes soil biological life with a focus on arbuscular mycorrhizal fungi (AMF) and its role in plant nutrient uptake, the establishment of agricultural Long-Term Experiments (LTEs) around the world, and the lessons that Canadian organic farmers and researchers can pick up from the rise and functionality of these LTEs. The second chapter investigates long-term manure effect on flax grown under organic management in a long-term field study. The study evaluates the effect of a long-term P mitigation strategy on P sufficiency and AMF infection in flax (*Linum usitatissimum* L.) grown in the 31st and 32nd year of a long-term field study in Manitoba. The objectives of the study are to assess: (1) Whether manure addition improves plant performance in terms of P tissue concentration, P uptake, AMF, and biomass; and (2) If changes in AMF related to plants P uptake. There were three hypotheses set for the study: (1) The organic manured treatment will produce higher flax biomass than the organic non-manured; (2) There will be less count of arbuscular mycorrhizal fungi abundance in the conventional treatment than in the organic; and (3) AMF will help to increase plant P tissue concentration. The third chapter assesses ‘Soybean response to novel organic amendments in a low phosphorus soil’. This study investigates various organic soil amendments as alternative sources of P to soybean grown in P-depleted soil (3ppm Olsen-P) under greenhouse conditions. The four organic nutrients used included composted beef cattle manure, anaerobic digestate, black soldier fly frass, and rabbit manure in natural-pellet form and ground form, which were compared to Mono-Ammonium Phosphate and a control (no amendments). The study’s objectives are to: (1) Assess different organic soil amendments as alternative sources of P to soybean grown in P-deficient soils; (2) Evaluate a novel manure source, rabbit manure, and compare its performance when in natural pellet form versus ground form; and (3) Test if AMF colonization affects the ability of soybeans to uptake P. I hypothesized that soybean will produce more biomass and P tissue concentration when planted in organic soil amendments than in the mineral fertilizer. My second hypothesis stated that any increase in AMF would result in greater soybean growth and P uptake.

1.1 The Evolution of Organic Nutrient Sources

As human populations expanded and land pressure grew, the traditional practice of allowing fields to lay fallow for extended periods to recover from nutrient depletion became less feasible. To address this challenge, the addition of organic materials emerged as a means to replenish lost soil nutrients and enhance crop yields. Some of the earliest soil amendments used by humans were organic in nature, primarily derived from wood and animals (including fish, birds, and livestock).

1.1.1 Wood Ash

Ash is the solid residue left after combustion of organic matter in biomass such as wood (Risse and Gaskin, 2013; Karltun et al., 2008). Wood-based ashes can be industrial (from industrial processes like the manufacture of pulp and paper) or non-industrial (from homes and wood-fired heat sources) (Azan et al., 2019). The early farmers used wood ash to enrich their agricultural soils from the 1700s through the early 1900s, and its application was considered a form of recycling, as it returned nutrients extracted from the land during harvest back to the soil (Risse and Gaskin, 2013). Today, wood ash is still used as a soil amendment and according to Schmidt and Naylor, (1986), hardwoods are preferred because they produce higher-quality ash containing approximately 6% potash, 2% phosphoric acid, and 30% lime.

Canada is one of the countries where timber, pulp and paper industries are the largest producers of wood ash that is used to amend forest soils (Hannam et al., 2018). The expansion of forests in Canada has increased wood ash production leading to its dumping in landfills as a waste material. The landfill dumping ignited reactions among individuals and researchers who viewed wood ash as a soil amendment especially for Canadian forest soils to replenish lost nutrients and promote closed nutrient cycles (Augusto et al., 2008; Kim et al., 2022). Wood ash is recommended as a forest soil amendment because it improves tree growth by stimulating the decomposition of soil organic matter to release nutrients to the tree roots and replenishes drained nutrient pools post tree harvesting (Huotari et al., 2015). The ash is rich in minerals like phosphorus, calcium, magnesium, and potassium, but contains very little nitrogen as most gets volatilized during the combustion process (Karltun et al., 2008). Additionally, wood ash counteracts the acidifying effects of forest soils after tree harvesting; as a liming agent with a pH range of 9 to 13, wood ash is a valuable alkaline component used to neutralize acidic soils and adjust pH to meet specific crop requirements (Risse & Gaskin 2013; Karltun et al., 2008). According to Huotari et al. (2015), tree harvesting depletes a lot of Ca in forest soils because Ca is highly concentrated in the bark and stem wood hence a lot of it is lost through timber harvesting. Tree species with relatively high Ca requirements like the sugar maple (*Acer saccharum Marsh*.) have shown signs of Ca deficiency (Kim et al., 2022) and addition of wood ash might resolve the issue by increasing the soil pH.

Despite its benefits, wood ash use as a forest soil amendment still faces certain setbacks in Canada. It is difficult to maintain a specific quality of wood ash since its chemical and physical properties vary depending on the source, tree species, and combustion method (Karltun et al., 2008). Application of wood ash in forests takes more time and money compared to landfill dumping hence a lot of it still goes to the landfills to avoid the economic costs. It is also challenging to obtain the necessary permits before applying ash to forests or agricultural soils due to potential environmental harm such as dust problems during application (Hannam et al., 2018).

1.1.2 Bird Guano

Guano is the excrement of birds (primarily seabirds) and bats that can serve as a natural plant fertilizer due to its nutrient content, mainly nitrogen and phosphorous (Schnug et al., 2018). In addition to the excrete, guano can also consist of eggshells and carcasses of dead seabirds. The hub of guano production was the Chincha Islands in Peru, primarily due to the high population of pelicans that consistently deposited their excrement on the islands. The Peruvian pelicans could accumulate guano up to 30 – 45 m tall (Schnug et al., 2018). According to Lesher (2008), the guano from the Chincha Islands was recognized for its high nitrogen content due to the island's hot and dry climate that kept guano dry for extended periods and secured its longevity and viability by inhibiting its bacterial breakdown. The seabird guano consists of 10–12% nitrogen, 10–12% phosphoric acid and 3% potash (Schnug et al., 2018).

Although no published papers on the current use of guano in Canadian organic farming were found, the Organic Federation of Canada has listed ‘dried deposits of guano’ as one of the permitted soil amendments for food production. It is further stated that the guano can be of wild bats or seabirds but must be left to decompose and dry adequately at the site of deposits before collection and application (https://organicfederation.ca/). The main challenges facing guano as a source of manure are: (1) Inconsistent quality as different factors including bird species, diet, and geographical location determine the quality of guano thus the great variations; (2) Guano has a slow nutrient release of P and reaches the peak 84 days after application (Hatibu et al., 2021). Mollah et al. (2020) advised that guano should be applied in soils at least two months before planting.

1.1.3 Fish Waste

Canada is considered to be one of the main fisheries countries that register large amounts of fish waste (Ahuja et al., 2020). There is a growing interest to recycle the increasing fish waste into organic fertilizers for farm use. Approximately 25% of the global amount of the total fish captured annually turns into fish waste; fish waste refers to the unwanted parts of the fish post-processing such as the intestines, tails and fins, skins, scales, and bones and can be 30–70% of the original fish (Muscolo et al., 2021). In an effort to promote circular economy by recycling nutrients from the sea back to the land, fish waste is used in production of fertilizers acceptable for organic farming (Ahuja et al., 2020). Commercial fish-based fertilizers are available in the market in many formulations such as bone meal, liquid fertilizer, and compost. Fish-based fertilizers usually contain significant amounts of N, P and Ca and especially fish bones that contain 90 to 123 g per kg P of DM, 140 to 239 g per kg Ca of DM, and 2 to 4 g per kg Mg of DM (Toppe et al., 2007). This is why fish-based fertilizers have been considered a potential source of P in organic farming considering more than 85% of P applied in organic agriculture comes from phosphate rock, a diminishing resource (Cordell & White, 2011).

Fish waste can be processed into different forms of fertilizers and using different methods depending on the fish material at use; it can be processed into liquid fertilizer, combined with other materials to produce fish compost or used as a substrate in anaerobic digestion (Ahuja et al., 2020). In Canada, OMRI-approved fertilizer products for organic farming with their respective N-P-K content include: Fish meal (10.1 - 4.5 - 0.5), fish bone meal (6 - 4.4 - 0.8), OMRI-certified Pacific Natural (2 – 3 - 0.3) (OMRI, 2020).

1.1.4 Livestock Manure

In addition to fish and bird manure, livestock manure too has a rich history in agriculture, with its use dating back to the 16th through the 19th century; cattle manure stands as one of the earliest forms of livestock manure to be used (Rayne & Aula, 2020).

1.1.4.1 Composted Beef Cattle Manure

More than 80% of the Canadian beef herd is raised on the prairie provinces of Alberta, Saskatchewan, and Manitoba (Pogue et al., 2018). Lethbridge County in southern Alberta poses as one of the most densely populated livestock regions in North America (Norris et al., 2023). Livestock farmers on the Prairies have embraced manure composting as a way of returning nutrients to the soil as well as an economic source for those who have excess and can sell it on the agricultural market (Eghball et al., 1997). The majority of nutrients present in the manure are derived from the cattle's dietary intake (Euken, 2009).

Cattle compost is a mixture of various ingredients; urine, feces, waste food, and bedding materials (Kuepper, 2003). When composted, cattle manure (the final product) varies in nutrient composition from the raw manure materials as shown in Table 1.1. The composting process as described by (Norris et al., 2023) includes: (1) “Manure is scraped into piles in the feedlot pens; (2) It is then loaded into a truck and deposited into individual compost windrows (10 m length, 2.5 m width, and 2 m high); (3) The windrows are turned 7–9 times for even decomposition; (4) The windrows are then rolled into curing piles and left for 3 months; (5) The compost is mature for use.” Several factors exert influence over the quality and characteristics of beef cattle manure, encompassing storage facilities, farm management practices, and environmental conditions (Eghball and Power, 1994). For instance, when cattle are reared in open lots, some of the nitrogen in the ammonia form may volatilize. Research done at the University of Nebraska found out that there was a 71% loss of N due to volatilization from earth feedlots during summer (Euken, 2009). Mitigating this nitrogen loss can be achieved by incorporating bedding or carbon sources into the manure during composting, effectively immobilizing the ammonia. The commonly used bedding materials for livestock in Canada include cereal straw and wood shavings, that also make part of the compost.

Table 1.1. Nutrient composition of raw material vs composted manure. Table adapted from (Ehsan et al., (2018).

Parameters	Raw material	Finished compost
Carbon (%)	31.3	20
Nitrogen (%)	1.30	1.9
C/N ratio	24.0	10.5
Phosphorus (%)	0.18	0.24
Potassium (%)	1.20	1.55
Iron (mg kg $^{-1}$)	520	630
Zinc (mg kg $^{-1}$)	42.0	54.0
Manganese (mg kg^{-1})	47.0	63.0
Copper (mg kg^{-1})	1.02	1.22

The composting and drying of cattle manure before application is encouraged to mitigate the presence of pathogens and weed seeds. Windrow composting of straw-based manure can reduce pathogen concentrations after 14 days of composting. Composting also destroys any viable weed seeds through the high heat environment (50° C to 70° C) and decomposition. In their experiment to test weed seed viability in beef cattle manure, Larney and Blackshaw (2003) observed that the rate of weed seed elimination was species-dependent; after 14 days of composting, green foxtail (*Setaria viridis*), redroot pigweed (*Amaranthus retroflexus*) and wild oat (*Avena fatua*) seeds had less viability to germinate of 4-6% compared to 73-88% in the control. By day 29 the viability dropped to 0.5-2%, and by day 70 viability was zero for all three species.

When applied to soils, cattle manure can increase or maintain soil C stocks because of its high C content. To test this statement, Liang et al. (2021) evaluated eight, long-term field studies across Canada assessing the agronomic performance of various types and rates of manure applications and their impacts on the SOC. He measured the Manure-induced Carbon Retention (MCR) coefficients in manured vs non-manured soil conditions and in liquid vs solid manure. The results showed that the solid cattle manure had greater MCRs than liquid manures (MCRs varying from 15% to 40%). Additionally, while solid cattle manure application had 36.5% MCR, a control (unfertilized) treatment had 19.6% while the synthetic fertilizer had 13,2%. In the same experiment but a different field in Ottawa, the decomposed cattle manure applied had a mean MCR of 25% compared with 12% of MCR from the fresh manure. Composting manure adds more C to it.

Cattle manure also improves the carbon sequestration efficiency as observed by (Balík et al., (2023) when he investigated the effect of long-term farmyard manure vs mineral fertilizer compared to unfertilized control. The application of farmyard manure increased carbon sequestration efficiency by 22.9%. According to (Meng et al., (2019) cattle manure can be used as a natural-based solution to combat salinization in agricultural soils under long-term management. Sodic soils, characterized by poor soil structure and low soil organic matter, are common in arid and semi-arid regions. In their experiment, Mao et al. (2023) planted maize (*Zea mays)* in two fields, one with annual manure application at a rate of 10,000 kg ha^{-1} yr^{-1} and the other one unmanured as control. The results indicated that long-term application of cattle manure to sodic soil resulted in increased soil porosity, water-holding capacity, and low bulk density in comparison to the control treatment. The manured soil had bigger water stable aggregates (0.5–1 mm) than the unmanured control (0.25–0.5mm) hence better porosity. Moreover, long-term application of cattle manure promotes soil available P at different soil depth. Manure application increased the proportion of soil labile-P, from 8%–13% at 0–20 cm depth and 5%–8% at 20–40 cm depth (Mao et al., 2023).

1.1.4.2 Rabbit Manure

Rabbit manure is a novel manure still finding its place in agricultural production. Rabbits (*Oryctolagus cuniculus*) stand out as remarkably productive domestic livestock due to their rapid growth and short gestation period of 30-33 days (Maharaj et al., 2006). Male rabbits reach breeding maturity at the age of 6-10 months, females reach at 5-9 months and the litter (offsprings) average ranges between 4-10 kits (Purdue University, 2020). As livestock, rabbits can be raised for different purposes namely meat, fur, wool, pets, exhibition and showing, and laboratory animals (Avesing and Judge, 2020). Renowned as "breeding machines”, their unique physiological traits, including mating-induced ovulation, brief gestation, and lactation cycles contribute to their impressive reproductive capabilities (Lebas et al., 1997). Importantly, due to their large waste generation comprising urine and droppings, rabbits are considered to be a promising source of manure.

Manure collection is mainly from the commercial breeds that feed on a specific diet and are large in size; the quality of manure from rabbits is highly dependent on the rabbit's diet. Normally, a rabbit will eat 3-4% of its body weight and should be fed twice a day, in the morning and in the evening at the same time every day (Avesing and Judge, 2020). On average, a full-grown commercial rabbit weighs around 4-5 kg (Parks, 2021). For quality manure production, the rabbit must be fed on a balanced diet comprising of: (1) Commercial pellets that are formulated with necessary nutrients; the pellets should contain 18-20% fibre, 16-18% protein, and 1% fat; (2) Hay such as timothy grass (*Phleum pratense*), oat, (*Avena sativa*), barley (*Hordeum vulgare*), alfalfa (*Medicago sativa*). (3) Fresh leafy greens including carrot tops (*Daucus carota*), dandelion leaves (*Taraxacum officinale*), mustard leaves (*Brassica juncea*), mulberry leaves (*Morus alba*), (Purdue University, 2020), and (4) Water; the water supply must be clean and constant; rabbits find it difficult to eat if they fail to drink water and this can reduce the general amount of organic waste generated (Avesing and Judge, 2020).

In Canada, rabbit production is modest and has been declining over the years (Figure 1.1). From the 2021 Census of Agriculture, there were around 1486 rabbit farms in Canada with most farms being in Ontario (Statistics Canada, 2024). A further report by Agriculture and Agri-Food Canada, (2021) shows the declining number of rabbit farms as well as head count. According to Lukefahr et al., (2004), the commercial meat rabbit industry in North America has been struggling and has failed to reach the level of success of other livestock species like cattle, swine, and chicken due to a range of obstacles especially a lack of demand for rabbit meat for human consumption, high labour demand, and insufficient experience/knowledge, that have inhibited the industry's progress. Lukefahr et al. (2004) further states that the solution chosen is to downscale rabbit production and make it a family-based enterprise mostly in rural/peri-urban areas for families' protein sustainability rather than commercialization for profit-making.

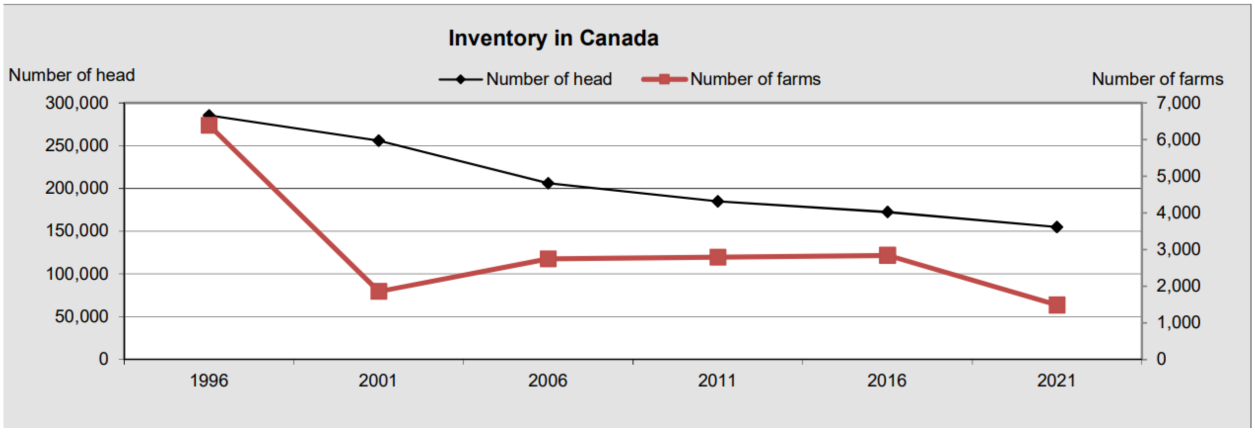

Figure 1.1. Rabbit inventory in Canada. Source: This figure has been adopted from Statistics Canada, Census of Agriculture

Rabbit manure stands out as an exceptional organic resource due to its elevated levels of nitrogen and phosphorus in comparison to other animal manure from sheep, goats, pigs, chickens, cows, and horses (Table 1.2). Its nutrient-rich composition has led to its informal name, "Bunny gold". As documented by Li-Li et al., (2013), the typical lifespan of a domestic rabbit ranges from 8 to 12 years, during which a single rabbit can produce approximately 28.8 kg of manure. In practical field scenarios, a recommended dry application rate of 20 t/ha^{-1} for rabbit manure has been established and to achieve this, around 700 rabbits would be required to generate the 20,000 kg of manure per hectare.

Table 1.2. Comparative NPK and organic matter content of various animal manures: Highlighting rabbit data. Table is adapted from Kuepper (2003), page 4.

Animal	% Nitrogen	% Phosphoric acid	% Potash	% OM
DAIRY COW	0.57	0.23	0.62	39.6
BEEF STEER	0.73	0.48	0.55	42.5
HORSE	0.70	0.25	0.77	69.7
SWINE	0.49	0.34	0.47	67.7
SHEEP/GOAT	1.44	0.50	1.21	53.0
CHICKEN	1.00	0.80	0.39	52.5
RABBIT	**2.40**	**1.40**	**0.60**	**65.0**

In addition to the manure, rabbit urine too is a nutrient-rich solution that promotes crop growth, owing to its abundant nitrogen and phosphorus content (Kemunto et al., 2022),. Furthermore, rabbit urine's ammonia content serves as a natural pest repellent deterring aphids, mites, moths and caterpillars and effectively acting as an organic insecticide (Kemunto et al., 2022). Each mature rabbit generates approximately 100-300 ml of urine daily, with higher nitrogen and phosphorus levels compared to other livestock. Rabbit urine contains 2.7% N, 1.1% P compared with cow 0.5% N, 0.2% P and sheep 1.5% N, 0.3% P (Indabo and Abubakar, 2020). The higher nitrogen levels in the urine have been attributed to a dominant forage diet with small volumes of water consumption.

The benefits of rabbit manure extend beyond its nutrient content. Unlike 'hot' manures such as cattle and chicken, which necessitate composting before application to prevent crop scorching, rabbit manure can be directly utilized in its natural pellet form (Rodelio and Honeylet, 2021). Nevertheless, prior to application, the pellets should undergo a drying process to eliminate any acidic urine stains that may burn crops. This trait has earned rabbit manure the label of a 'cold manure,' characterized by its non-burning effect on crops. This versatile manure can be employed in various forms (top-dressing or composting) throughout the crop's growth period. Diluting rabbit urine with water at a 1:3 ratio creates an organic foliar nutrient beneficial for crops (Maharaj et al., 2006), particularly leafy vegetables like spinach, cabbage, and lettuce, as well as grains such as corn, wheat, barley, and sorghum.

Rabbit droppings can also be used in vermicomposting as an excellent food for worms that convert organic waste into nutrient-rich compost (Rodelio and Honeylet, 2021). Vermicomposting is the process in which the worms feed on, break down the rabbit droppings and release it as worm poop, commonly referred to as vermicompost. Vermicompost has a higher rate of diverse bacteria that are beneficial to the soil and plants (Saranraj, 2012). The enzymes and the microbial population in the compost enrich plants. Improved microbial activity not only breaks down more organic matter to release nutrients, but also fights harmful pathogens, safeguarding the plants' growing environment (Piya et al., 2018). Additionally, plants can more readily take up nutrients from that soil because they have been broken down and made available in plant-form. Rabbit vermicompost is known to have a higher N, P, K content compared to other types of vermicomposted manures: Rabbit vermicompost: 1.64% N, 1.90% P, 2.45% K, 18.65% C, goat manure 1.26% N, 1.35% P, 0.33% K, 25.23% C, buffalo manure 1.21% N, 1.06% P, 0.38% K, 15.83% C, and cattle manure 0.74% N, 0.78% P, 0.18% K, 15.90% C (Rodelio and Honeylet, 2021). Rabbit vermicompost is considered to be the best version of rabbit manure because it provides beneficial bacteria and enzymes to the soil. Worm beds are usually placed beneath hanging rabbit cages to ensure a continuous food supply as shown in Figure 2. Although rabbit cages can be made from different materials including wood, iron sheets and welded wire mesh, the welded wire mesh has been recommended to be the best. Rabbit cages' floors should therefore be made of wire mesh and suspended as demonstrated in Figure 1.2, to allow the droppings and urine to pass through to the ground for its collection. The problem with all-wood cages is that many rabbits in such cages are susceptible to ear mites and the wood can also rot especially when in constant contact with the rabbit urine or water. The iron sheets on the other hand attract a lot of heat which is unfavourable for the rabbits and can get extremely cold in winter affecting especially the kits (Szendro et al., 2016). The ratio recommended is 2-3 rabbits per 1000 worms to provide sufficient sustenance for the worms (Webster, 2015). Some of the worm species used in vermicomposting include the red worms (*Eisenia foetida)* for cold climates and African Nightcrawlers (*Eudrilus eugeniae*) for tropical climates (Webster, 2015).

The primary obstacle to embracing rabbit manure is its scarcity, stemming from limited production and insufficient understanding of its utilization. In contrast to larger livestock, rabbit production tends to be smaller in scale. Additionally, research on rabbits as both livestock and sources of manure for organic farming is lacking, leaving many farmers unaware of its potential benefits.

Figure 1.2. Worm beds placed beneath hanging rabbit cages to directly collect rabbit droppings which are food to the worms. The figure is sourced from Pinterest https://www.pinterest.com.au/pin/185351340885563492/

1.2 Emerging Alternative Organic Nutrient Sources

As organic agriculture expands across Canada, the need and interest to explore other sources of phosphorus, in addition to livestock manure, intensifies. Insect frass and food waste are among the alternative P sources being experimented in Canada for use in sustainable agriculture.

1.2.1 Frass

Insect farming has captured the interest of organic agriculture due to its dual role in transforming organic food waste into natural plant fertilizer and providing protein for both human and livestock diets (Gärttling and Schulz, 2022). Additionally, due to their small size, insects require less land for production compared to other large, manure-producing livestock like cattle and swine hence a lower negative environmental impact (Nicksy and Entz, 2021b). Manure produced by insects is referred to as frass. Frass is the biological residue or excreta produced by an insect as a natural outcome of its metabolic processes (Van Looveren et al., 2022). In Canada, frass is mainly derived from Black Soldier Fly (BSF) (*Hermetia illucens*) larvae that is fed on a specific diet of non-meat food waste from food stores and restaurants (Nicksy and Entz, 2021b). The BSF consumes food waste, digests and processes it, and expels it as a valuable end-product. The reuse of food waste as insect feed contributes to a circular economy in agriculture (Amorim et al., 2024) . Frass from BSF has been recognized as the most nutrient-packed; BSF has total N (3%), K (4%) and increases seed germination by (>90%) (Amorim et al., 2024). Nicksy and Entz, (2021b) also used BSF frass in a pot study with Italian ryegrass to measure and compare plant P uptake from the frass treatment compared with a non-fertilized control and MAP treatment; the frass treatment showed the highest P uptake and this was attributed to the high nutrient content of frass (32 g/kg^{-1} N, 8.7 g/kg^{-1} P, and 8.7 g/kg^{-1} K).

The BSF can be fed on various non-meat organic materials, including grass cuttings, fruits, and vegetables. Consequently, the quality and nutrient content of frass vary and are highly influenced by the specific food substrate provided during the rearing process (Klammsteiner et al., 2020a). The insect gut has various bacterial communities whose enzymatic activities perform two important roles: (1) they eliminate toxic compounds such as pesticide traces from the foods consumed by the insect and (2) they break down the cellulose present in plant tissues consumed and facilitate the insect to assimilate simple sugars (Poveda, 2021). This is why insect frass is considered to have fast mineralization with its nutrients easily assimilated by the plant roots. This observation was supported by Choi et al. (2009) when they compared the growth rate of Chinese cabbage grown in BSF frass and a commercial fertilizer and found no significant differences. BSF frass performed just as well as the commercial fertilizer. Poveda (2021) also added that insect frass has a faster decomposition rate because it contains more labile C, which can stimulate the activity of microbial decomposers. In addition to the type of food material used being a quality determinant, its nutritional content also matters. A nutrient-rich diet will produce a similar frass product and vice versa. Kagata and Ohgushi, (2012) assessed the effects of frass quality on the soil N availability and plant growth, using frass of cabbage moth (*Mamestra brassicae* L.) that fed on fertilized and unfertilized mustard spinach *Brassica rapa* L. var. *perviridis* Bailey. The results showed that the frass excreted by larvae that fed on the fertilized plants had higher N than that of the insect fed on unfertilized plants. Frass derived from insects fed on N-rich plant leaves had a higher N than frass from insect on an N-poor diet.

Critical to its efficacy is the accurate application rate of BSF frass, which dictates crop performance. To test both the effect of application rate and nitrification on N uptake from insect frass, Watson et al., (2021) did a pot trial with Italian ryegrass to understand N release from frass, plant growth rate and nutrient uptake using mealworm frass, applied at two rates (1.5 and 3% w/w). They observed that the “3% w/w rate inhibited seed germination, possibly due to salinity or ammonia toxicity,” while the 1.5% rate worked efficiently and led to higher biomass and N uptake. Similarly, Ramesh et al. (2009a) investigated the application rate and the resulting effect of BSF frass on corn using a pot study. In their experiment, he used BSF: soil ratio of 1:2 which resulted in stunted plant growth, an indication of phytotoxic due to high proportions of frass and its high ammonium concentration to soil.

Although some studies have stated that insect frass is safe for use because the insect gut has bacteria capable of destroying any toxins from the food intake (Poveda, 2021), some still have speculations especially because frass has a higher microbial count that may act as a potential harbor of pathogens including Salmonella, which could pose risks to consumer health. To counteract this concern, Van Looveren et al. (2022) tested and recommended frass heat treatment at 70 °C for 60 minutes as a solution to mitigate potential harm while retaining frass's characteristics as a natural fertilizer. Economic considerations further hinder frass widespread application, as the production and collection of insect excreta remain complex.

1.2.2 Anaerobic Digestate

Food waste contributes significantly to environmental degradation, posing a challenge in its sustainable management for households, food industry, and processing plants. Globally, around 32% of food produced transforms into waste during various handling stages and most of it ends up in landfills as a pollutant (Mickan et al., 2022). According to Gooch et al. (2010), Canada is no different, with approximately 40% of all its produced food turning into waste negatively affecting the country's economy and environment. Biodigesters seem to be a promising solution to the problem as they divert food waste from landfills. Food waste include municipal and agricultural wastes that tend to be rich in proteins, minerals, and sugars, which can be reused as raw materials in compost making for farm production; this will create a sustainable way of recovering valuable nutrients via anaerobic digestion to achieve circular nutrient use in agriculture (Wainaina et al., 2020).

Anaerobic digestion, a biological process, involves the degradation of biodegradable organic material such as food waste or livestock manure by microorganisms in the absence of oxygen, resulting in the production of biogas and natural plant fertilizer (Nicksy and Entz, 2021b). Biogas, composed mainly of methane (70%) and carbon dioxide (30%) (Mickan et al., 2022), is an environmentally friendly fuel for domestic use, characterized by smoke-free combustion which curtails air pollution. Moreover, during anaerobic digestion, organic N is transformed into ammonium, which subsequently undergoes rapid nitrification within the soil, converting it into plant-accessible nitrate-N.

The output of the anaerobic digestion process comprises liquid and solid fractions, both of which are useful in agriculture. The liquid fraction, enriched with nutrients, serves as an alternative nutrient solution for hydroponic production, especially for green vegetables. Notably, research has indicated that dilution of the liquid fraction from food waste in a 1:5 ratio with water, increases foliage yield and plant growth in hydroponic systems (Fuldauer et al., 2018). Conversely, the solid fraction undergoes composting and is frequently dried and pelletized for convenient application and transport. Solid fractions typically exhibit a greater concentration of phosphorus than their liquid counterparts (Nicksy and Entz, 2021b). The pretreatment given to food waste has a huge effect on the quality and characteristics of the end-product (digestate). Tampio et al. (2015) compared the characteristics of autoclaved and non-autoclaved digestates from food waste and observed that the autoclaved digestate had 40% less microbial activity as microbes could not adapt to the conditions within the autoclave. The autoclaved digestates showed low ammonification and ammonium nitrogen content and as a result, solid fractions from autoclaved food waste would be more suitable as fertilizer for leguminous crops because of their low nitrogen but higher potassium and phosphorus levels.

Hallat-Sanchez et al. (2023) evaluated the impact of anaerobic digestate on plant growth by conducting a pot experiment growing spring barley (*Hordeum vulgare*) in three treatments: 120 kg N ha^{-1} digestate, a synthetic fertilizer, and unfertilized control. When plant height was measured, there was an increase of 7–20% with anaerobic digestate compared to control due to the increased availability of N to the crop. In another study, Ross et al. (1989) carried out a 6-year investigation on the impact of anaerobic digestate as a fertilizer for crop growth and comparisons were made among three treatments: anaerobic digestate, a synthetic fertilizer, and a water-only treatment, using three crops, maize, oats, and kale, over a 2-year rotation. As per the results, there were no significant differences between treatments in the final yields and N concentrations were observed higher (exact figures not given) in the anaerobic digestate and synthetic fertilizer treatments than in the water-only treatment. From these experiments, anaerobic digestate influenced plant growth more than the control and performed similarly to the synthetic fertilizer demonstrating its potential as an organic soil amendment.

While the application of digestate can be as a standalone or mixed substance, it is particularly effective when used in isolation. Mickan et al., (2022) investigated the response of tomato (*Solanum lycopersicum*) plants to digestate alone vs digestate + biochar in a potted experiment of 45 growing days. Shoot biomass and N concentration were measured; the results showed higher dry shoot mass in digestate-alone (1.91 g) than in the digestate + biochar mixture (1.85 g). Similarly, the digestate-alone had significantly higher shoot N concentrations than the digestate + biochar (no exact figures were given). Although application of biochar has some positive effects to the soil including reducing soil bulk density and increasing the water holding capacity, it has a negative effect on N mineralization rate: biochar can maintain the inorganic N content in the soil thus decreasing the N mineralization (Fu et al., 2019).

Potential nitrogen losses through volatilization and odor-related problems are some of the limitations that restrict the broader implementation of digestate as a nutrient source. Additionally, economic challenges including high production and transportation costs frustrates its marketability. Organic solid waste treatment requires heavy infrastructural investment for the purchase of necessary technologies that may include mechanical, thermal or biological treatments processes (Wainaina et al., 2020). However, in countries like Costa Rica, low-cost infrastructural material is used by majority of the farmers to recycle their farm waste which mainly includes kitchen waste, livestock manure, banana and pineapple wastes. As Arias (2009) explains, the digesters are made from tubular PVC membrane bags culturally called “sausage digesters” and they are preferred due to their affordability (Figure 1.3). He adds that, “the digesters are installed in the ground and measure 20 m long, 2.5 m diameter and can hold 32,500 gallons of liquid volume and 2,700 cubic feet of biogas accumulation chamber.”

Figure 1.3. Biogas installation with tubular PVC membrane bags in Costa Rica.

1.3 Combining Organic Manure Sources for Farm Fertilization

Due to the long-term negative effects of conventional farming including air, water, and soil pollution, organic agriculture has been a recommended as a safer alternative for reversing the damage and sustaining soil management and environmental well-being (Cen et al., 2020). Sustainable soil management can be defined as, “the capacity of soil to function within ecosystem boundaries to sustain productivity, maintain environmental quality, and promote plant and animal health" (Herrick, 2000). Adoption of organic farming saw an increase in the use of manure from various sources such as animals, crop residue, food waste, insects, and earthworms. Organic manures help in the improvement of soil structure, aeration and water holding capacity of soil (Subramanian et al., 2020). Additionally, the manures improve soil and crop quality, yield, and farmers' profit in the long run. Several factors influence crop response to manure including the manure's nutrient release rate, crop's nutrient requirement, and the type of manure and its nutritional composition (Ali et al., 2020). Manure can be applied alone or in combinations and different studies have been carried out to evaluate the difference in effect caused especially on crop productivity and nutrient uptake.

Ali et al. (2020) did a 2-year field study to investigate the influence of different organic manures and their combinations on productivity and grain quality of bread wheat. The manures used were sugarcane press mud (residue of the filtration of sugarcane juice), vermicompost, and farmyard manure compared to a control (no manure). The results indicated that vermicompost + farmyard combination gave the highest protein (12.2%) while the lowest protein (10.6%) was found in the control, vermicompost alone had the highest gluten (24.05%) and starch (59.9%). The economic analysis showed that farmyard manure alone gave the highest net profit (USD \$532 ha^{-1}) compared with vermicompost alone (\$234 ha^{-1}). Ali et al. (2020) further stated that the economic analysis was evaluated mainly based on manure's cost of production and economic yield as farmers focus most on profit maximization. The highest profitability was achieved through farmyard manure as compared to vermicompost and press mud because the production cost of farmyard manure is lower than the other two. Combining different manures can improve certain plant growth parameters as illustrated above and this could be due to the multi-nutrient supply from the manure combo. For a majority of the parameters, vermicompost alone performed better than when in combination. A possible reason for that could be that vermicompost nutrients are readily available to plants, but when mixed with farmyard manure for example, its efficiency lowers. Additionally, in comparison with conventional compost, nutrients can be retained for longer periods in vermicompost because it has better water holding capacity and porosity from the humus concentration (Ali et al., 2020).

A similar study was carried out by Ramesh et al. (2009) to evaluate the effect of combining different organic manures (cattle, poultry, and vermicompost) compared with mineral fertilizers on the productivity of soybean, durum wheat, chickpea, and mustard grain. The results found were interesting: Mineral fertilizer recorded the highest durum grain yield (4.622 Mg ha^{-1}), cattle + vermicompost + poultry manure recorded the highest yield of soybean (1.039 Mg ha^{-1}), chickpea grain yield was the highest with cattle manure + vermicompost combination which was similar to mineral fertilizers and the mustard-grain yield was the highest in the mineral-fertilizer (1.922 Mg ha^{-1}). Ramesh et al. (2009) further stated the crop's N requirement was the main determinant in its response to the various amendments. Durum wheat and mustard had lower yield in organic-manured plots compared to the mineral-fertilizer treatment because they are high N-consuming crops, so the slow nutrient release from the organic sources starved them of nutrients thus the low growth and yield under organic and higher under mineral treatment. Cattle + vermicompost + poultry manure resulted in higher soybean yield among the other combinations due to fast and adequate release of nutrients from the organic manures particularly poultry and vermicompost at initial stages.

Combining organic manures is not necessary unless the result is expected to be better than its individual performance. From the experimental results mentioned above, certain organic manures such as vermicompost and poultry can be considered high yielding due to their fast release of plant-available nutrients. These manures perform good (increase general plant growth) when used alone but can also improve the quality of a second manure, if combined.

1.4 The Role of Phosphorus in Organic Systems

1.4.1 Phosphorus Crisis in Canadian Organic Farms

According to the report by COTA (2018), organic farming in Canada is on the rise with approximately 5,791 certified organic producers across the country. Although the individual organic acreage has not increased, the total land size under organic management expanded to 1.3 hectares, representing 2.1% of Canadian agricultural land (Lynch, 2022). The increase in organic farms sparked discussions on nutrient management of such systems to maintain stable yields. Nutrient management addresses the addition, removal, and recycling of nutrients from the farm and in organic systems, nutrient sources are mainly carbon based (manure, compost) and non-processed mineral sources like rock phosphate (Nelson and Janke, 2007). Phosphorus fertilization has become of particular interest in organic agriculture due to its necessity in crop production as well as its limited availability.

Various on-farm studies conducted on Canadian organic farms have indicated phosphorus deficiency. In a report from 14 organic farms surveyed in Manitoba, Saskatchewan, and North Dakota, Entz et al. (2001) observed that the farms were sufficient in other nutrients like nitrogen (N), potassium (K), and sulfur (S), but often deficient in P. Additionally, the average P level recorded in their study was 15 kg ha^{-1}, lower than that usually found in Manitoba agricultural lands, 20 kg ha^{-1} (Knight et al., 2010). A study done at Glenlea, Manitoba, showed that after 8 years of organic production, the alfalfa grown in the wheat-alfalfa-alfalfa-flax rotation began showing signs of P deficiency with undermined productivity (Carkner et al., 2020). Similarly, Welsh et al. (2009) reported that in the absence of P fertilization, the organic plots at the same Glenlea study, became P deprived after 13 years. Canadian organic farms, mostly grain farms, lose more of their phosphorus reserves through farm export practices like harvests without having a matching P import mechanism and according to Martin et al. (2007), the standard soil tests performed on some of the long-term organic farms on the Canadian Prairies, especially grain farms, presented low P values. Addition of soil amendments whether organic or inorganic to a cropping system is necessary. Even when combined with other sustainable farming practices such as crop rotation, long-term cropping systems with no additional soil nutrients will result in nutrient deficiencies and low yields (Rutunga and Neel, 2006). Effective P fertilization methods should balance the P inputs with crop removal.

1.4.2 The Role of P in Plant Growth

Phosphorus is an essential macro nutrient needed for plant growth and development and it is vital for various plant activities including germination, photosynthesis, respiration, and seed formation (De Silva et al., 2015). However, despite its importance, its availability in the soil for adequate plant uptake is often limited and P deficiency is a common concept affecting 42% of the global cultivated land (Mwende, 2019). Majority of the plants suffer from P starvation because they can only absorb the free inorganic phosphate from the soil but whose mobility is often restricted hence inaccessible (Y. Lu et al., 2023). Another possible reason for P limitation is that while other fundamental elements like carbon, nitrogen, oxygen, and hydrogen can circulate freely in the atmosphere, phosphorus cannot because it has no significant gaseous phase (Cordell and White, 2011). Ensuring long-term availability and accessibility of phosphorus sources is critical for global food security.

Due to its importance and constant deficiencies especially in low input organic systems, a lot of research has been and continues to be carried out to identify the best way to add and manage P in the agricultural system. One of the biggest worries for researchers is the global dependent on P mined from the phosphate rock, a non-renewable source that not only faces extinction but also poses environmental harm through its mining (Cordell and White, 2011). This is why renewable and organic sources of P such as crop residues, food waste, animal/insect excrete are being encouraged. Aside from reliable P sources, the environmental and soil conditions also influence the capacity of plants to uptake P. Soil characteristics including pH, moisture, nutrient level, and microorganism community strongly determine P use efficiency (Chtouki et al., 2022).

1.5 Soil Biological Life

The health of the soil and its microorganism's composition constitute the bedrock of organic farming; soil microorganisms include microbiota (bacteria, fungi, protozoa, and small nematodes), mesofauna (mites, springtails, larger nematodes) and macrofauna (larger insects, earthworms, burrowing vertebrates) (Schonbeck et al., 2019) Research on the below-ground microorganisms targets to explore relationship between biological life in the soil and its impact on soil fertility and crop productivity. This includes investigations into the role of microorganisms like Arbuscular Mycorrhizal Fungi (AMF) in enhancing phosphorus plant uptake

1.5.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular Mycorrhizal Fungi (AMF) are soil-borne microorganisms that establish mutually beneficial relationships with plant roots, facilitating the transfer of nutrients from the soil to the plants (Dejana et al., 2022). AMF facilitates nutrient uptake by absorbing nutrients from the soil and transferring them to host plants via intraradical structures called arbuscules (Dirks and Jackson, 2020). The symbiotic relationship formed involves plants providing carbon to the fungi in exchange for improved plant nutrient uptake, especially phosphorus (P) (Cheeke et al., 2013; Antunes et al., 2012). Most plants are mycorrhizal and it is estimated that AMF form associations with more than 80% of flowering plant species (Nguyen et al., 2019). When the symbiotic relationship is formed, AMF provides P from soil to host plants through their hyphal networks expanding the root's surface area for enhanced nutrient uptake (Dirks and Jackson, 2020). Nutrient exchange between plants and fungi primarily occurs in structures called arbuscules; arbuscules are "branched clusters of hyphae that grow between the plant cell wall and cell membrane, serving as the primary sites for nutrient exchange between the plant and fungus" (Welsh, 2007b). Approximately 90% of the phosphorus present in plants is sustained by AMF ((Crossay et al., 2017).

While AMF naturally occur in the soil, they can also be introduced using commercial inoculants. Increasing phosphorus uptake in low-input systems can be achieved by either introducing exotic AMF strains through inoculation or preserving the native AMF communities in the soil (Li, 2016; Hudi et al., 2014). However, AMF inoculation is not guaranteed and its success depends on three key factors: (1) Species compatibility - the introduced inoculant must establish functional symbiosis with the target plant species; (2) Field carrying capacity - the soil habitat must align with the introduced inoculants; and (3) Species competition – there should be no negative interactions between the new inoculant and local AMF communities (Li, 2016a). Mäder et al., (2000) compared the rate of AMF colonization between plants inoculated vs natural population of AMF. The results showed colonization rates of 8% - 14% of root length under natural AMF population and 24% and 39% under AMF-inoculated. Upon inoculation the root colonization was much higher. This could possibly indicate that the level of the natural population of AMF in the soil could be below the required amount for adequate colonization.

Although the principal role of AMF is nutrient uptake, they also provide other benefits to their hosts namely pathogen protection, drought tolerance, and water acquisition (Dirks and Jackson, 2020). AMF activates plant defense responses when it senses an attack on the host plant's root system, acting as a bio-protectant against soil pathogens. Kim, (2017) investigated strawberry (*Fragaria ananassa*) production and observed that farmers preferred planting rootstocks rather than seeds because the former have higher disease tolerance, quicker growth, and better fruit quality. However, to maintain successful growth and production, the rootstocks were AMF-inoculated before planting to boost their immunity against soil pathogens (Kim, 2017). It is therefore advisable for farmers, especially those who run nurseries to incorporate AMF more into the soil to provide robust rootstocks that rely more on AMF than fungicides for pathogen protection. AMF also helps the plant absorb more water hence contributing to higher yields. According to Bista et al. (2020), plants assign more energy on developing a relationship with AMF more under drier than wetter conditions because they need extra help to reach soil moisture at deeper soil levels. This observation is also supported by Eziz et al. (2017) who found out that under drought situations, plants invest more in developing a root system than the shoot because a deeper root network will facilitate better water uptake from belowground while less aboveground growth will minimize water loss through transpiration. AMF root colonization increases the root's surface area to reach for more moisture in the soil rhizosphere.

1.5.2 Factors Influencing AMF Colonization

Although many biotic and abiotic factors influence mycorrhizal colonization, according to Dejana et al. (2022), the three most influential factors are host plant, nutrient availability (especially phosphorus), and environmental conditions.

1.5.2.1 Host Plant

Including AMF host plants in crop rotation systems is essential. Most plants are compatible with AMF except for members of the Brassicaceae family, such as cabbage, kale, mustard, and canola (Li, 2016a). Since AMF associates with more than 80% of flowering plant species, diversifying crops within a rotation can enhance AMF abundance and diversity, as diverse hosts support a richer AMF community of approximately 270 known species (Crossay et al., 2017; Nguyen et al., 2019). Growing non-mycorrhizal hosts like canola in a rotation can reduce subsequent AMF diversity and abundance, potentially affecting nutrient uptake in the following crop. In a study, Hall et al. (2016) stated that when flax was grown following wheat (a mycorrhizal crop), its root biomass and AMF root colonization was higher than when grown after canola, (a non-mycorrhizal).

1.5.2.2 Phosphate Inhibition Mechanism of AMF Colonization

According to Antunes et al., (2012), the relationship between mycorrhizal fungi and the host plants can be mutual or parasitic depending on the fertilization and chemical characteristics of the soil. Although application of P in sufficient amounts promotes plant growth and provides plenty of carbon for AMF, the host plant receives almost no benefit in terms of AMF-mediated P uptake. P sufficiency varies with the plant's age as presented in (Table 1.3) (Chakraborty and Prasad, 2019) and under these P-sufficient conditions, two scenarios can occur: (1) the plant can limit AMF colonization to conserve its sugars for growth and reproduction, or (2) the AMF can be available but solely as consumers, potentially leading to parasitism (Shao et al., 2023; Antunes et al., 2012). When the phosphorus concentration is low (<0.15%), AMF have a symbiotic relationship with plants but when the phosphorus concentration is high (> 0.70%), AMF can only become consumers, thus resulting in a parasitic relationship that limits plant growth (Shao et al., 2023).

The sufficiency range of P in plants varies depending on the plant species, plant parts, and growth stage. P deficiency in plants can be visually identified at the early vegetative phase and the common sign is the reddish-purple color that appears along the edge of the lower plant leaves (Chakraborty and Prasad, 2019). To determine the amount of P absorbed by the plant, a plant tissue analysis can be carried out to check whether there is sufficiency, deficiency, or extra range.

Table 1.3. The sufficiency range of phosphorus for various crops. Adapted from (Chakraborty and Prasad, 2019)

Crop	**Growth stage**	**Plant part used for tissue testing**	**P sufficiency range (%)**
Corn	Seedling (<10 cm height)	Whole plant	0.40%-0.60%
	>10 cm height to tasseling	Most recent mature leaf	0.30%-0.50%
	Tasseling/bloom	Ear leaf	0.25%-0.50%
	Maturity	Ear leaf	0.25%-0.40%
Small grains (barley, oat, rye, wheat)	Seedling to tiller	Whole plant	0.20-0.50%
	Jointing to flag leaf emergence	Top 2/3 leaves	0.20%-0.50%
Soybean	Flag leaf to maturity	Flag leaves	0.20%-0.50%
	Early growth	Most recent mature leaf	0.30-0.60%
	Flowering	Most recent mature leaf	0.30-0.60%
Cotton	Early bloom	Uppermost mature cotton leaf	0.20%-0.65%
	Late bloom/maturity	Uppermost mature cotton leaf	0.15%-0.60%
Canola	Prior to flowering	Recently mature leaf blades	0.42%-0.69%
Peanuts	All growth stages	Aboveground plant	0.20%-0.50%
Alfalfa	Bud to 10% bloom	10-15 cm of the plant	0.25-0.70%

Bermuda	Before heading	Upper half of the plant	0.20-0.40%
Fescue	Before flowering	Aboveground plant	0.26-0.40%

1.5.2.3 Effect of Soil Moisture Content on AMF

Different levels of soil moisture influence AMF colonization differently because the fungi express behavioral changes at certain soil water conditions. In an experiment carried out by Shukla et al., (2013) to measure AMF abundance at three soil moisture levels (field capacity, half field capacity, & double field capacity), maximum AMF colonization was recorded at field capacity. Soil moisture on both extreme ends can impair AMF colonization. As a result, reduced AMF infection was observed at low moisture level (half field capacity) and at excess moisture (double field capacity).

Extreme drought causes very low moisture levels that limits the development of intra-radical structures such as arbuscules and vesicles (Shukla et al., 2013). Hence longer and extreme drought lowers AMF infection rate. However, there is a certain drought level (not identified yet) that provokes the extension of the mycorrhizal external mycelia for better water absorption. On the other hand, excessive wet or flooded conditions also lower AMF colonization because flooded conditions create anaerobic conditions or increase P availability in soil which decreases AMF (Cavagnaro, 2016;Shukla et al., 2013).

1.5.2.4 Effect of Soil pH on AMF

Soil pH is an important factor for plant growth as it regulates nutrient availability as well as the activities of soil microorganisms. However, since most nutrients are readily available in neutral soils, a majority of microorganisms tend to reside and thrive in neutral soils with pH 6.5-7.5 (Soti et al., 2015). This observation was as well supported by Carrino-Kyker et al. (2016) who investigated the response of AMF to the experimental elevation of soil pH in temperate hardwood forests. When the soil pH rose to between 6.5 – 7.5, AMF root colonization was almost double the amount in low soil pH (pH 5.5 and below). Rising soil pH altered AMF abundance. Similarly, Soti et al. (2015) conducted a study to understand the effect of soil pH on the mycorrhizal colonization of climbing fern (*Lygodium microphyllum)* in a 60-day greenhouse pot experiment; the plants were grown in pots filled with pH-adjusted soils ranging from pH 4.5 to 8.0. The results showed that

AMF root colonization was higher in soil pH 5.5–7.5 and lowest for plants growing in pH 4.5 or 8.0. Therefore, increase in AMF colonization can be associated with increasing pH to a certain value.

1.6 The Rise of Agricultural Long-term Experiments (LTEs)

In the face of a swelling global population, the agricultural sector struggles to ensure ample and safe food provision. Consequently, the field of agriculture has evolved into a dynamic space, where researchers and experts persistently undertake experiments to understand the various determinants of food production. Amidst the shock and pressure of mounting climate change combined with the harmful effects of conventional agriculture on air, soil, water, and biodiversity, the call for embracing 'sustainable agriculture' has intensified (Hiranandani, 2010). Yet, the realization of this aspiration has encountered complexities, particularly due to the influence of diverse factors, prominently environmental ones such as rainfall and drought. The essence of sustainable agriculture rests on meeting current needs while safeguarding the capacity of future generations to meet their own requirements. Attaining this equilibrium necessitates 'sustainable agriculture' to: (1) uphold consistent annual agricultural yield in terms of both quantity and quality; (2) offer economic viability for farmers and interconnected stakeholders; and (3) foster the preservation of soil and environmental well-being (Johnston & Poulton, 2018).

One important way to understand and design sustainable agriculture for food security and soil health is to study systems over an extended period of time. This goal has led to the establishment of many agricultural Long-Term Experiments (LTEs) around the world. LTEs refer to enduring agricultural trials that are focused on studying soil health and crop yield over a minimum span of two decades (Grosse et al., 2021; Rasmussen et al., 1998). One of the significant advantage of LTEs over short-term studies is that their prolonged observational window accounts for the variable nature of environmental conditions (Macdonald et al., 2020). According to Grosse et al., (2021) there are five major characteristics that an agricultural experiment must fulfill to be classified as a long-term experiment: (1) The research should have a minimum duration of twenty years; (2) The focus of the research should at least include soil properties and crop yield; (3) The setup of each trial should allow for statistical analyses by having replications and clearly defined factors and experimental design; (4) The farm practices such as crop rotations, soil amendments, weed management, and tillage should be consistent; and (5) The farm's features such as the soil type, management system (conventional or organic), plot size, number of plots, treatments, and replications must be stated.

The first LTEs sprung up mid-late 19th century and initially embarked on simple experiments comparing plant responses to synthetic and organic nutrients. An example is the Rothamsted Experiment Station, a collection of experiments, established by Sir John Lawes and Sir Henry Gilbert (Jenkinson, 1991; Johnston & Poulton, 2018b). One of the earliest Rothamsted experiments, the Broadbalk winter wheat, started in 1843 under three treatments: Unmanured, fertilized with N, P, K, Mg and fertilized with farmyard manure (Jenkinson, 1991). The main objective of the experiment was to test the importance of the plant nutrients N, P, K, and Mg on crop yield and to compare organic vs inorganic nutrients (Moss et al., 2004). After three years of the experiment, they observed signs of N deficiency in the unmanured plots which significantly lowered yields to 1.3 Mg ha^{-1} compared to the fertilized (2.5 Mg ha^{-1}) and farmyard (2.8 Mg ha^{-1}) treatments (Jenkinson, 1991). As food production systems evolved in complexity, so too did LTEs, transitioning from the study of singular crop-input responses to embracing analysis of multi-component systems (Macdonald et al., 2020; Norris et al., 2023). According to Johnston & Poulton (2018), industry researchers and food producers became more interested in the establishment and analysis of LTEs with five objectives in mind: (1) To evaluate the sustainability of the selected farming systems and their resilience to the changing climatic conditions; (2) To collect and build a database on best management practices that benefit farmers and the environment; (3) To archive samples of soil and plant material for further scientific research; (4), To reach a more realistic assessment of the effect of agricultural practices on the environment; and (5) To provide long-term datasets for mathematical models that describe different agricultural practices.

Long-term studies can be used to study P uptake and utilization through different strategies such as intercropping, soil amendments/fertility, and preservation of soil biological life. An et al. (2024) assessed four intercropping systems of chickpea/maize, faba bean/ maize, oilseed rape/maize, soybean/maize in a 12-year long-term field experiment to measure shoot P content. The results showed that the intercropping enhanced shoot P content by 49.4% compared to the monocultures. An example of a beneficial intercrop given by An et al., (2024) is pigeon pea/cereal as "pigeon pea can use iron-bound P, while intercropped cereals rely on calcium-bound." This illustrates how intercropping can allow access to different P fractions to promote P uptake in soils. Long-term studies apply different soil amendments including organic and conventional to maintain soil fertility and crop yields. Th level of soil fertility maintained can have a direct effect on the AMF population present and subsequent plant nutrient uptake. Shao et al., (2023) observed that AMF abundance lowered when P was added to the soil, whether organic or synthetic; the addition of P raised the soil P level which limited AMF-plant symbiotic relationship as the plant under high P concentrations may not need extra help accessing P. Soil biological life influences both the underground and aboveground growth of plants. Different crop species/rotations and soil amendments applied in long-term studies can influence how P is added to and consumed from the soil. Although AMF have the ability to reach and transport more P from deeper soil depths to plant roots, they must be compatible with the intended crop; including AMF host plants in crop rotation systems is therefore essential. Growing non-mycorrhizal hosts like canola in a rotation can reduce subsequent AMF diversity and abundance, potentially affecting nutrient uptake in the following crop. In a study, Hall et al., (2016) states that when flax was grown following wheat (a mycorrhizal crop), its AMF root colonization was higher than when grown after canola, a non-mycorrhizal.

1.6.1 Establishment of LTEs in Canada

Currently, Canada boasts 17 officially recognized Long-Term Experiments (LTEs) managed jointly by federal government bodies and esteemed academic institutions such as the University of Alberta, Guelph, and Manitoba, among others (Norris et al., 2023) To enhance collaboration and knowledge exchange, the 17 Canadian LTEs united under the banner of the North American Project to Evaluate Soil Health Measurements (NAPESHM) with the aim of fostering synergy among researchers to facilitate in-depth investigations on soil management practices. As the initiative evolved, its research horizons broadened, encompassing an array of subjects ranging from crop rotation, cover crops, soil amendments, soil erosion, tillage, and the development of drought-tolerant crop varieties (Norris et al., 2023) as shown in Table 1.4. These LTEs can be classified into three main groups of static, semi-static, and dynamic (Loughin et al., 2007). Static LTEs have a fixed research component from their inception where the treatments, input rates, or crop rotation stay consistent. Although static LTEs offer consistency, their rigidity can be a hindrance to the adoption and representation of current physical or biological changes in the soil. Semi-static LTEs, in contrast, exhibit a degree of flexibility; their core research objectives remain intact, but adaptable adjustments are made to account for necessary and time-relevant evolving farming practices. Dynamic LTEs, as the name suggests, pivot in real-time to stay aligned with the modern changes of farming practices (Loughin et al., 2007). However, the dynamic nature of such experiments poses challenges in recording historical research data for long-term reference. The Glenlea LTE studied in this paper is an example of semi-static and is the first and currently the oldest organically-managed LTE rotation study in Canada, established in 1992 (Entz et al., 2004).

Table 1.4. Examples of agricultural long-term experiments established to investigate different research topics in various Canadian provinces

Name of LTE	Year founded	Location (Province)	Initial research question(s)
Breton Plots	1929	Alberta	What crop rotations and fertilizers could improve agricultural production on Gray Wooded soils?
Stavely Research Ranch	1949	Alberta	What are the effects of long-term cattle stocking rates on the Dark Brown Chernozem soil?
Elora Research Station	1980	Ontario	What are some of the most productive and profitable crop rotation options for corn farming?
Swift Current 0MC (Zero, Minimum, and Conventional Tillage Study)	1981	Saskatchewan	What is the effect of tillage and crop rotation on soil quality and crop production under semi-arid, rain-fed conditions of the Canadian prairies?
Glenlea Study	1992	Manitoba	Can crop rotations reduce external farm inputs, particularly synthetic products?
Ridgetown Long-Term Cover Crop	2007	Ontario	What cover crops perform best in a vegetable–grain rotation?

1.6.2 Lessons from LTEs that might Inform Organic Cropping in Canada

The LTEs are a legacy of practical experience that continue to act as a guideline for researchers and food producers in the agricultural industry. There are a lot of historical agronomic data from LTEs ranging from soil fertility and amendments, crop performance, weeds, pests, diseases, and soil biological life, that have been collected and archived for reference and can be of value to current organic farmers in Canada.

Established in 1843, the Rothamsted experiments still serve scientists and researchers who occasionally analyze the historical stored soil and plant samples to understand nutrients under different agricultural management systems (Jenkinson, 1991). Similarly, organic researchers in Canada could collect and archive soil and plant samples that can be studied by future generations to understand patterns in yield and nutrient dynamics under organic agriculture. Additionally, the current agronomic data being collected including biomass, yield stability, soil pH, water use efficiency, weed population, AMF abundance and diversity should also be archived for future comparisons. Second, it is vital to record and archive challenges facing Canadian organic farmers including nutrient deficiency, yield reduction, soil acidification (Berti et al., 2016) and the solutions implemented for reference by others. Third, yield stability is essential especially for organic producers who expect both quality and quantity in harvests to recover their earlier investment. It is therefore valuable to monitor yield stability in organic management under the changing environmental conditions and keep records to inform the decisions of other organic farmers. Historical yield data could also be used as evidence for the ever-going debate that usually associate organic farming with low yields and conventional with high yields.

LTEs study mainly soil characteristics and crop performance over an extended period of time. Some of the common soil properties investigated in earlier established LTEs like the DOK trial included soil biological life and their influence on nutrient uptake especially P. Other parameters studied by LTEs include greenhouse gas emissions. All these are measurements are relevant to organic farming especially soil biological life which largely dictate the success of organic systems. Some of the findings from certain LTEs like the Rothamsted experiments are very applicable to organic farmers in Canada. For example, Johnston and Poulton, (2018b) stated that in one of the experiments, the inorganic fertilizers yielded similar to farmyard manure for wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and sugar beet (*Beta vulgaris*). Such positive results on manure motivate organic farmers and cancel the stereotype that organic agriculture equals to low yields. Moreover, Johnston and Poulton, (2018b) carried out an experiment to measure soil organic matter in barley that was fertilized with 35 kg P ha$^{-1,}$ 90 kg K ha^{-1}, and 35 t ha^{-1} farmyard manure. The results showed that the soil with farmyard manure contained 2.5 times more soil organic matter (SOM) than the treatment with fertilizers. This is an observation and an encouragement to organic farmers that organic soil amendments build more SOM than conventional fertilizers, which subsequently increases the microorganism population due to food availability. SOM also improves soil texture facilitating plants' access to nutrients and water to boost yield.

1.7 Previous Research

Investigations on long-term agricultural experiments, soil biological life with a focus on AMF, and P deficiency in Canadian organic systems have been done by various researchers in the Natural Systems Lab at the University of Manitoba. The present study joins many of such studies; some of the studies include, "Mycorrhizal colonization of flax under long-term organic and conventional management" (2004) whose objective was to observe the influence of conventional and organic management systems on AMF populations in flax under two different crop rotations in the 12th year of Glenlea Study (Entz et al., 2004). In 2007, Welsh, (2007b) investigated, "Organic crop management can decrease labile soil P and promote mycorrhizal association of crops" to determine the influence of different cropping systems on soil (rego black chernozem) P levels. In 2011, Kirk et al., (2011) looked at "Mycorrhizal colonization, P uptake and yield of older and modern wheats under organic management" with the aim of evaluating AMF colonization, tissue P and grain yield of five modern (post 1990) and five older (pre1970) spring wheat cultivars over 4 site-years in Manitoba. In 2021, Nicksy and Entz, (2021a) added to the research by assessing, "Recycled nutrients as a phosphorus source for Canadian organic agriculture: a perspective" to provide an introduction to the importance of recycled fertilizer sources in the global P cycle, and the key role they can play on organic farmland in Canada. In 2021, Nicksy et al., (2021) followed with "Recycled nutrients supply phosphorus and improve ryegrass yields on phosphorus-depleted soil" a greenhouse experiment that compared three sources of recycled P — struvite precipitated from municipal wastewater, black soldier fly frass from food waste, and anaerobic digestate of food waste — to mono-ammonium phosphate (MAP), compost, and a control. The present study introduced rabbit manure as a new organic amendment to the lab's investigation. There is still need for researchers to explore more organic nutrients to diversify options for organic farmers.

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2. Manure Effect on Flax Grown under Organic Management in a Long-term Field Study

2.1 Abstract

Phosphorus (P) deficiency is one of the main challenges facing organic farms on the Canadian Prairies and lasting solutions are needed. This study evaluated the effect of a long-term P mitigation strategy on P sufficiency and arbuscular mycorrhizal fungal (AMF) infection in flax (*Linum usitatissimum* L.) grown in the 31st and 32nd year of a long-term field study in Manitoba. Flax was planted after wheat (*Triticum aestivum* L.) in a 4-year – alfalfa (*Medicago sativa* L.) – alfalfa – wheat rotation under three treatments: organic manured (ORG M), organic non-manured (ORG NM), and conventional (CONV). The manure blend (80 kg/ha P) applied was a mixture of equal parts black soldier fly frass, anaerobic digestate of food waste, and beef cattle compost, added once per rotation cycle in the fall of the first alfalfa year. Conventional plots received fertilizers to soil test recommendations each year. Flax was sampled at the flowering phase; after 52 days and assessed for above-ground biomass, P tissue concentration, plant P uptake, and AMF root colonization. The individual questions tested from the study were: (1) Did manure addition improve plant performance in terms of P tissue concentration, P uptake, AMF, and biomass; and (2) Were changes in AMF related to plants P uptake. The ORG M treatment produced higher biomass than CONV and ORG NM. Within organic production, the addition of manure increased biomass by 33%. ORG NM flax presented higher P tissue concentration and AMF abundance than the ORG M, 8% and 22% higher, respectively. AMF abundance was observed as ORG NM > ORG M > CONV where a decreased abundance of AMF was observed when P was added to the soil. Manure addition to flax seemed to increase the plant's growth rate but not its P tissue concentration nor AMF abundance. However, flax amended with manure had a higher AMF colonization than where mineral fertilizer was used.

2.2 Introduction

While most conventional farms in North America face excessive levels of soil P due to high inputs, organic farms face soil P shortages, according to the standard soil test measurements (Martin et al., 2007). Canadian prairie organic grain farms, for example, consistently indicate deficiencies in available soil P and the question of interest is, why do most Canadian organic farms show a P deficit? First, there are limited organic sources of P that are acceptable based on the organic certification standards; some of the organic P sources available to farmers include livestock manure and phosphate rock (Carkner et al., 2023). These sources come with certain limitations: phosphate rock has a slow release of P especially in high pH soils common on most organic farms in Canada while animal manure is unavailable to most farmers and if purchased off-farm, has a high price and transportation costs (Aziz et al., 2013). Secondly, most organic farms on the prairies, especially grain farms, export P in grain harvests but have minimal P adding options, and third, limited extension services on the alternative (non-livestock) sources of organic P have barred many farmers from exploring other options. The outcome of these challenges has been consistent P insufficiency levels on organic farms across Canada. Entz et al., (2001) reported that in a survey done, 170 organic fields in Manitoba were surveyed and the available P levels were low, ranging from 4 to 54 kg ha^{-1}, with long-term organically managed farms for over 30 years having the lowest values. Additionally, 73 fields in Saskatchewan that had been managed organically for at least 5 years showed similar low P levels (Knight et al., 2010).

Despite P unavailability being a common characteristic on many Canadian organic farms, farmers still report decent yields and this could be due to the soil biological activities, such as AMF, that aid in supplying the crop with the available P; Braman et al. (2016b) compared soil biological activity between the annual grain and forage-grain rotations under organic and conventional management at Glenlea long-term study and observed higher activity in organic, especially in the forage-based rotation. According to Welsh, (2007a), “the ability of crops to grow on soils low in available phosphorus while unavailable reserves are made available may determine the sustainability of organic systems”.

AMF are soil microorganisms that enhance plant’s nutrient uptake by colonizing the roots of the host plant to form a mutual relationship (Wahab et al., 2023). For mutual benefits, AMF penetrates the root’s tissue to form mycorrhizae, an exchange spot for nutrition where AMF provide P to the plant in return for C for its energy and growth (Y. Lu et al., 2023). AMF possess external hyphae that can absorb phosphate from the soil and convert it into polyphosphate compounds; the polyphosphates are then broken down into organic phosphates absorbable by plant cells (Hudi et al., 2014). P efficiency of a plant increases with the increase in AMF abundance. (Rahayu et al., 2021) investigated the of AMF inoculation on P uptake of soybean and when compared, the AMF inoculated soybean had higher P uptake effect average (0.53%) than the non-inoculated (0.50%). Similarly, Welsh, (2007a) also compared AMF colonization between the organic and conventional systems at Glenlea and found out that the percentage AMF colonization as arbuscules and spores was higher in organic than conventional systems.

Flax is an important crop in Canada grown mainly for its oil (flaxseed contains 20-40% oil) used in industrial production of varnishes, inks, cosmetics, and paints (Zhang et al., 2011). Additionally, the human consumption of flax seed is increasing because of its high dietary fiber, omega-3 fatty acids, anti-carcinogenic properties, and its use as a vegetable oil (Keene & Dakota, 2020). Presently, various edible forms of flax can be found in the food market, ranging from whole flaxseeds, milled flax, and roasted flax. Consumer demand for flax products is growing due to the health benefits including high protein content varying from 20% to 30 % with gluten-free properties (Goyal et al., 2014). The Canadian Prairie (Manitoba, Saskatchewan and Alberta) is the major flax growing area in Canada (Siemens, 2019;Jhala & Hall, 2010), due to the region's favorable temperature range of 10 to 25°C during the growing season (Hall et al., 2016). This has positioned Canada as the world's largest producer and exporter of flaxseed (Lafond et al., 2007;Agriculture and Agri-Food Canada, 2009). The increasing concerns of consumers for their health has encouraged adoption of organic flax seed as the demand for food products rich in omega-3 oils (Zhang et al., 2011) continue to rise. Generally, flaxseeds are available in two basic varieties: brown and yellow or golden (Goyal et al., 2014), and farmers are advised to plant the certified seed for good seed viability and minimal weed content (Keene and Dakota, 2020).

In the present study, flax was selected as the most suitable crop in the grain-forage rotation of flax – alfalfa – alfalfa – wheat to study the crop's response to manure and the relationship between AMF root infection and P uptake. Flax was selected for this research for a few reasons: First, flax is considered a great host for AMF for its reliance on AMF-plant root symbiosis relationship for increased soil P uptake (Y. Li et al., 2019). Due to its weak root system, flax capacity to absorb nutrients and water is limited to approximately the top 70 cm of soil (Agriculture and Agri-Food Canada, 2009; Hall et al., 2016); it thus relies heavily on AMF colonization to extend its root system for more nutrient uptake. Second, flax is known to thrive in low phosphorus organic systems that largely benefit from the association of AMF to offset P unavailability (Welsh, 2007a), unlike soils with heavy P applications that impede AMF function (Shao et al., 2023).

Third, flax tends to have an unexpected response to manure addition. To maintain P levels on organic farms, farmers apply organic-approved P sources, namely, composted manure and rock phosphate and practice legume-flax rotation to encourage N fixation (Y. Li et al., 2019). Despite the addition of amendments, phosphorus application is not recommended for flax production because flax shows no yield response to added phosphorus fertilizer/manure (Keene and Dakota, 2020). It appears that the flax plant prefers uptake of soil residual phosphorus originating from P fertilization of preceding crops (Iowa State University, 2008), compared to the direct application of manure or a high-rate P fertilizer.

Flax in the current study was grown in weed-free (weeds were manually removed) subplots rotated with other crop species, namely wheat and alfalfa, that are AMF hosts. This was to eliminate the challenge of weed pressure and AMF non-hosts plant species that could have limited the intended assessment of AMF effect on the growth and P uptake of flax. Flax is said to be one of the most difficult crops to grow organically because of its poorly developed root system which makes it less competitive against weeds. Flax's poor resistance against weeds has been linked to lower its AMF abundance and subsequently its P uptake. In a study conducted in flax organic plots at Glenlea rotation by (Carkner et al., 2020), weed competition was observed to be greater in the manured compared with the non-manured treatment because weeds responded better than flax to the added nutrients. The dominant weed specie in the flax was wild mustard which is a non-mycorrhizal plant and whose dominance over flax reduced the population of AMF host plants (Jordan et al., 2000). Hall et al. (2016) also confirms that the presence of a non-mycorrhizal host can reduce AMF abundance, potentially affecting nutrient uptake.

This study investigated 'Long-term manure effect on flax grown under organic management in a long-term field study'. In the grain-forage rotation of flax – alfalfa – alfalfa – wheat, flax was selected as the crop of interest because of its unique and unexpected response to addition of P amendments, its capacity to thrive in low input systems and reliance on AMF for nutrient uptake under different soil conditions. The main objective was to assess the effect of manure on flax that was grown under three treatments: organic manured (ORG M), organic non-manured (ORG NM), and conventional (CONV), in a long-term field study. The individual questions tested were: (1) Did manure addition improve flax performance in terms of biomass, P uptake, and AMF colonization? and (2) Did AMF help flax access P? The first hypothesis was that the organic manured treatment would produce higher flax biomass than the organic non-manured. Secondly, it was hypothesized that there would be less count of arbuscular mycorrhizal fungi abundance in the conventional treatment than in the organic. The third hypothesis stated that AMF would help to increase plant P tissue concentration.

2.3 Materials and Methods

2.3.1 Site Description

This study was conducted in two field seasons (Apr-Oct 2022 and 2023) at the Glenlea long-term rotational study located 20 km south of Winnipeg, Manitoba, Canada, (49.39 N and 97.7 W) on Treaty 1 territory. The soil is Rego Black Chernozem comprised mainly clay (55%), silt (32%), sand (12%), and organic matter (5.5%) (Carkner et al., 2020). Average temperature over the duration of the growing seasons 2022 and 2023, was 13.5 °C and 13.8 °C respectively. Total precipitation for the 2022 growing season was 685.2 mm and 321.7 mm for the 2023 growing season.

Glenlea occupies a distinctive niche as a semi-static Long-term Experiment (LTE) as it has progressively adopted evolving changes in its crop rotations, agricultural inputs, and experimental design while maintaining its original objective. Initially, the study was implemented to evaluate how crop rotations could curtail the need for added farm inputs, particularly synthetic products (Carkner et al., 2020). The trial's experimental design was a split plot randomized complete block, and the main plots had three, four-year crop rotation treatments: (i) Grain-only rotation (wheat-pea-wheat-flax), (ii) green manure-grain rotation (wheat-clover-green manure-wheat-flax), and (iii) forage-grain rotation (wheat-alfalfa-alfalfa-flax) (Welsh, 2007b). Both rotations were grown under conventional and organic management.

However, in 2003, the Glenlea study underwent some modifications. Its experimental design changed from a split-plot randomized complete block to a completely randomized block format, incorporating all rotation phases annually. The original three four-year crop rotations evolved into two types: (i) Grain-only rotation (flax-oat-soybean-wheat) and (ii) grain-forage rotation (flax-alfalfa-alfalfa-wheat), both still under both conventional and organic management (Carkner et al., 2020). Green manure, clover, and peas were substituted with oats and soybeans in the crop species. Further modifications related to farm inputs were implemented in 2007;

composted beef cattle manure was introduced and integrated into the organic plots and all organic plots were split into manured and unmanured treatments (Braman et al., 2016b). This supplementation aimed primarily at raising P levels in organic soils which had significantly dropped (Welsh et al., 2009). N was supplied via legume-driven N fixation. Weed management strategies were also revised in 2011, shifting from postemergence harrowing to interrow cultivation, reducing potential crop damage. The interrow cultivation, which involves a specialized implement with a backswept knife that eliminate inter-row weeds, is undertaken when the crops reach approximately 15 cm tall. The 2-m pathway between the plots gets regularly tilled to reduce weed encroachment. Some of the common weeds in the organic plots include Canada thistle (*Cirsium arvense* L), wild mustard (*Sinapis arvensis*), and green foxtail (Setaria *viridis*).

In 2020, the manure addition underwent further changes shifting from solely beef cattle compost to a combination of equal parts cattle compost, anaerobic digestate, and black soldier fly frass. The current design also includes a restored native grass prairie in each of the three replicates which is burned every 4 to 5 years, in late spring (end of May). Presently, this investigation was carried out within the grain-forage rotation. The rotation encompasses flax (*Linum usitatissimum* L.), a two-year forage cycle with alfalfa (*Medicago sativa* L.), followed by wheat (*Triticum aestivum* L.), grown under organic manured, organic non-manured, and conventional treatments. Flax was the crop of interest in the rotation.

2.3.2 Experimental design and Treatment Application

A Completely Randomized Design with three replicates was used in the study. There were three treatments: Organic Manured (Org M), Organic Non-Manured (Org NM), and Conventional (CONV). The conventional plot size was 28 m x 4 m and the organic one was 14 m × 4 m. The length of the organic treatments was half that of the conventional because the organic was split into two equal parts to accommodate manured and non-manured sub-treatments. The manure was added once per rotation cycle in the fall of the first alfalfa year. All plots were separated by a 2-m path and the study was fully phased such that plots for each crop in the cropping sequence were present every year.

2.4 Data collection

2.4.1 Manure and Soil Analysis

All manure samples underwent analysis at Agvise Laboratories in North Dakota to determine their nutrient content (Table 2.1).

Table 2.1. Nutrient analysis results of the manure blend (frass + digestate + cattle compost) used as soil amendments in the organic plots at the Glenlea study.

Measurement	Frass	Digestate	Compost	Unit
Total Nitrogen	3.2	3.8	0.71	%
Total Phosphorus	0.874	2.753	0.227	%
N:P ratio	3.7	1.4	3.1	
Potassium as K2O	2.1	0.97	1.2	%
Sodium	0.44	0.43	0.1	%
Calcium	0.25	6.9	6.3	%
Ca:P ratio	0.29	2.5	27.8	
Magnesium	0.52	1.2	2.1	%
Zinc	84	360	140	ppm
Iron	871	18125	5852	ppm
Manganese	87	369	295	ppm
Copper	12	96	21	ppm
Sulfur	0.35	1.4	0.14	%
Water Content	9	8	18	%
Carbon %	40	35	8.9	

Table 2.2. Amount of P added to the organic plots with the addition of manure each year. The manure blend is added at the rate of 80 kg/ha P

	Proportion (%)	Kg P/ha from manure blend	Kg N/ha from manure blend	Kg manure/plot
Frass	30	24	88	13
Digestate	40	32	44	6
Cattle compost	30	24	228	38
Total	100	80	360	57

2.4.2 Soil Sampling and Analysis

Soil sampling was conducted in the fall preceding the flax crop and again in the subsequent summer after the crop was sampled for biomass, AMF, and P uptake. Within each treatment, soil samples were obtained from marked quadrats (0.25m²). Each treatment's replicate had two quadrats, resulting in a total of six samples per treatment. A long-handled shovel was used to extract the soil cores using the following steps: (1) The shovel was pushed into the ground at an angle to achieve the desired 15 cm depth. (2) The shovel was tilted back to scoop full blades of soil which were then placed in labeled plastic zip bags and transported to the laboratory. After each sample, excess soil from the shovel was removed by hand to get subsamples with approximately equal amounts of soil across all depths. (3) At the laboratory, soil samples were homogenized and passed through a 4-mm sieve and subsamples were air-dried and shipped to Agvise Laboratories for analysis (Table 2.3).

Table 2.3. Soil basic nutrients in the experimental area for 2023 growing season. Each treatment took three soil samples and the average value was calculated. Soil samples were taken in July after flax was sampled. All results were rounded to one significant figure (all values are the mean)

Treatment	Phosphorus (P) (ppm)	Potassium (K) (ppm)	Nitrate(N) (lb/acre)	pH
CONV	28.7	622.5	36.2	6.6
ORG M	20.7	682.3	22.7	7.7
ORG NM	6.8	593.7	20.7	7.6

2.4.3 Biomass

Above-ground flax biomass was collected at the crop's full flower reproductive stage. Two, 0.5 m x 0.5 m quadrat samples were taken from each treatment using a hand-held sickle cutting to ground level. All the plants within the quadrat were sampled. The samples were then put in labeled drying bags and dried in the oven at 65°C for a minimum duration of 72 hours.

2.4.4 Phosphorous Tissue Concentration

Once dried and weighed, the flax biomass samples were ground using Thomas Model 4 Wiley Mill Machine and passed through a 1 mm sieve. These finely ground samples were carefully placed in plastic snap-cap vials, ensuring a minimum of 1 g per vial, and were then sent to Agvise Laboratories in North Dakota, USA, for complete nutrient analysis. The lab used the wet digestion method for plant tissue analysis with Inductively Coupled Plasma – Atomic Emission Spectrometry using nitric acid as described by (Havlin and Soltanpour, 1980).

2.4.5 Flax Root Sampling for AMF

Root sampling took place at the reproductive (full flower) phase of flax after 48 and 52 days of growth in 2022 and 2023, respectively. Two sampling points within the marked quadrats were chosen per treatment. At each point, approximately five to ten flax roots and the encompassing soil were carefully dug out to a depth of 15 cm using a shovel. The roots, along with adhering soil, were placed in labeled plastic zip bags and kept cool in ice-filled coolers during transportation to the processing lab. At the lab, the roots were further submerged in water for at least one hour and gently washed free of soil. After thorough rinsing of the roots under tap water, fine root segments measuring at least 1.5 cm were gathered from each treatment. They were then temporarily stored at 4 °C in 50% ethanol for further assessment of AMF colonization.

2.4.6 Assessing AMF Root Abundance

The evaluation of AMF root abundance in flax roots was conducted at the Soil Science Laboratory of the University of Manitoba. The aim of the procedure was to identify and count the number of arbuscules present in the prepared roots. The Magnified Intersections Method (MIM) described by Mcgonigle et al. (1990) was used to process and count the AMF. As a method, MIM requires the roots to be observed at a magnification 200x which is sufficient to identify the presence of arbuscules.

The assessment of AMF involved four major steps: Step one was *root preparation* - the roots were delicately removed from ethanol and placed into specialized cassettes. They were then rinsed with Reverse Osmosis (RO) water and placed within 100 mL glass beakers, (RO water is water whose ions and molecules have been removed). Step two was *clearing* - the glass beakers containing the cassettes and roots were immersed in a 10% KOH solution to remove tannins in the roots. Next, the beakers were gently covered with aluminum foil and autoclaved for 8 min. After autoclaving, the roots underwent additional rinsing with tap water and acidified water. Step three was *staining* - the roots were returned to the beakers, covered with an ink-vinegar solution, and gently boiled for 3 min on a hot plate. Following boiling, the roots were rinsed repeatedly with acidified water, and they were stored in the same solution to preserve their condition. Step four was *mounting* - the stained roots were placed on a petri dish, and a few drops of glycerin were added to facilitate dispersion. The roots were individually mounted on microscope slides and were aligned parallel to the long axis of the slides (25 segments of 1 cm each per slide).

Cover slips were added to the mounted slides, gently pressed to remove moisture, and left to dry before counting the arbuscules. The slides were observed at a 200x magnification. The counting of AMF was done as explained by Kim, (2017). The root segments were examined by going up and down the slide from the top left corner of the slide towards the bottom right corner of the slide. Each visible and countable root strand was considered one intersection and the counting was done till 100 intersections. Some of the roots were not counted either because the dye did not absorb well and they were too dark or the root was too thick. If the root contained arbuscles, hyphae, or vesicles, it was counted as AMF being present.

2.4.7 Data Analysis

Data were analyzed using the statistical analysis software program, R and were checked for normal distribution using the Shapiro-Wilk test. The experiment was laid out in a completely randomized design as adopted by the field study in 2003 when it underwent some modifications in its experimental design from a split-plot randomized complete block to a completely randomized design, incorporating all rotation phases annually (Welsh, 2007b). The experiment had three treatments and three replicates where the treatments were considered fixed effects and replicates the random effects. Difference among the parameters (dry matter biomass, P tissue concentration, P uptake, and AMF colonization) were tested using Analysis of Variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT), where means were considered statistically significant at $P<0.05$. Additionally, linear regression analysis was conducted to define the relationship between AMF colonization and plant P tissue concentration.

2.5 Results and Discussion

2.5.1 Biomass

A comparison of flax biomass in the main treatments at Glenlea study are shown in Figure 4. Significantly higher biomass was observed in the CONV and ORGM treatments. For example, flax in the ORG M treatment produced the highest shoot dry weight (188.7 g/m^2), followed by the CONV (180.5 g/m^2), and lastly the ORG NM treatment, (114.9 g/m^2). Biomass production was lower across all the treatments in 2023 compared with 2022 due to the drier soil conditions. Precipitation averaged at 97.9 mm/month in 2022 but only 45.9 mm/month in 2023 (Figure 2.1). No significant differences in flax biomass were observed in 2023.

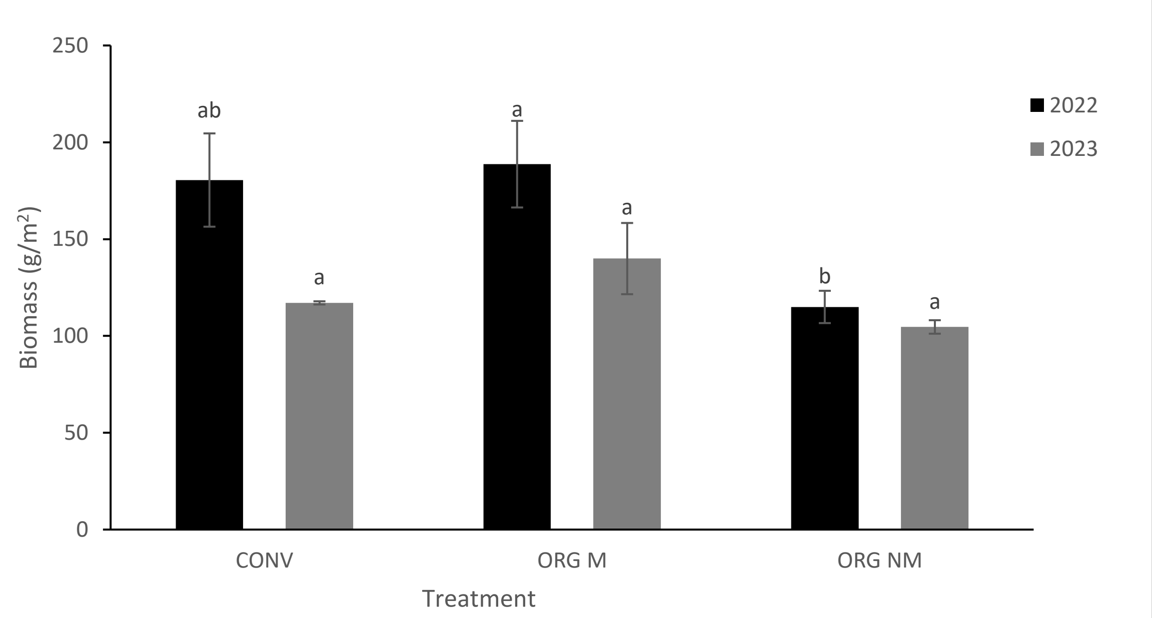

Figure 2.1. Flax biomass at flowering phase from the conventional (CONV), organic manured (ORG M), and organic non-manured (ORG NM) treatments during the 2022 and 2023 planting seasons at the Glenlea long-term rotational field study. Samples taken in the month of July for both years. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the P<0.05 level, according to the Duncan's Multiple Range test (DMRT). Significance compared between treatments per year.

Table 2.4. Monthly historical data report for total precipitation (mm) and mean temperature (°C) for 2022 and 2023 growing seasons. The report was derived from the Winnipeg station (nearest location to Glenlea study), positioned at Latitude 49°55'00.000" N, Longitude 97°14'58.000" W, with an elevation of 238.70 m. (Source - https://climate.weather.gc.ca/).

	April	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Total**	**Average/ month**
Monthly precipitation (mm)									
2022	118.2	166.2	116.0	85.8	115.1	36.3	47.6	685.2	97.9
2023	15.5	20.5	74.3	66.3	40.2	28.8	76.1	321.7	45.9
Mean temperature (°C)									
2022	-0.3	11.2	17.4	20.0	19.0	14.2	6.4		13.5
2023	1.1	15.8	21.0	17.8	18.4	16.2	6.4		13.8

Two main factors influenced flax growth in the present study; drought and soil P fertilization regime. Drought-like conditions experienced in 2023 reduced flax shoot dry weight. Drought influence on biomass allocation differs between the shoot and root. In a meta-analysis that investigated the impact of drought stress on biomass allocation, Eziz et al. (2017) found that under drought situations, plants adapt by reducing the aboveground growth to minimize water loss through transpiration. Additionally, plants respond to water scarcity by decreasing their stomatal activity to minimize water loss which consequently lowers photosynthesis and general shoot growth (Bista et al., 2020). The minimal effects of AMF on plant growth at low water may indicate that under these dry conditions, AMF colonization does not strongly contribute to nutrient and water acquisition.

Different sources of P have varying influence on the growth of plants. Jing et al. (2019) compared the availability of P from mineral fertilizers (NPK) vs animal manure to maize in the Askov long-term experiments, Denmark. The experiment measured plant height and dry weight and the results showed almost similar measurements for NPK and animal manure amendments. Average plant height was 182 cm (NPK) and 196 cm (animal manure) while the dry weight was 51.3 g (NPK) and 52.0 g (animal manure). Based on the results, it appears that under long-term management, animal manure improves crop performance either similar or better than mineral fertilizers and this could be attributed to the manure's residual nutrient effects, especially N and P, from previous manure applications. Unlike chemical fertilizer, organic amendments provide more plant-available P in the long run through organic P mineralization as the organic amendments are broken down by microbial and enzyme activities (Lu et al., 2020). Organic manures also add C to the soil and help in the improvement of soil structure, aeration and water holding capacity of soil (Subramanian et al., 2020). Manure's positive effect on crops increases with time.

Nutrients from manure and fertilizer increased crop biomass especially under the wet conditions of 2022. The application of these soil amendments raised soil P levels where CONV had 28.7 ppm and ORG M, 20.7 ppm compared to the ORG NM (no amendments) with only 6.8 ppm. These P additions increased aboveground growth; ORG M and CONV produced 39% and 36% more biomass respectively than ORG NM. The observation coincided with the global meta-analysis by Hou et al. (2020) who reported that P additions increased aboveground biomass by 13.9% in croplands (no specific plant species were mentioned in the study). In another study, the influence of P addition on the growth of barrel medic (*Medicago truncatula)* plants fertilized with different P concentrations (P0, P20, P50) mg P kg^{-1} was studied. Shoot dry weight from plants grown in P0 averaged at 0.5 g, P20 at 1.5 g while P50 weighed 2.0 g (Nguyen et al., 2019). The results showed that the shoot dry weight increased with increasing soil P addition.

Antunes et al. (2012) investigated the long-term use of specific soil fertilization treatments on the growth of barley (*Hordeum vulgare*). The shoot dry weight of barley significantly dropped when grown under P deficient conditions compared to sufficient conditions. The addition of fertilizer was consistent with the increase in shoot dry weight; the treatment without P addition produced less dry weight (0.82 ± 0.060 g) than the one fertilized with N, P, K, Ca (1.01 ± 0.11f g). In another similar experiment, Dejana et al. (2022) compared 3, P fertilization levels ranging from limiting to sufficient P (0.3, 0.7 and 1.0 mM) to evaluate the effect that will have on the growth of tomato plant. The results revealed that the shoot dry weight increased with the addition of P as indicated: 0.3 mM P was the most limiting thus lowest average weight, 20 g; 0.7 mM P followed with 30 g while 1.0 mM P presented almost 40 g shoot dry weight. Based on the above examples, it can be concluded that the soil P level has a significant impact on plant growth.

Although flax yield data was not presented in this experiment, some studies show that the relationship between biomass production and the yield potential of a crop tends to be linear (Li et al., 2023). However, the relationship can differ especially when comparing different genotypes of crop species or when the environmental conditions are not optimum (Long et al., 2006). Yield potential refers to "the yield of a cultivar when grown in environments to which it is adapted, with non-limiting nutrients and water, and with pests, diseases, weeds, lodging and other stresses effectively controlled" (Long et al., 2006). According to Duvick (2005), yield increase in grain crops like maize (*Zea mays*) can be attributed roughly 50% to genetic improvement and the other 50% to improved farm management practices. Increased above-ground growth can be linked to higher photosynthesis which is associated with higher yields. However, this observation cannot be generalized as photosynthesis can be limited by sink capacity; the ability of a crop to use photosynthate. After flowering, some grain crops experience a decreased sink capacity where the number and potential size of the seed formed lowers (PEET and KRAMER, 1980). As a result, the visually seen biomass does not translate into the expected yield. As a solution, breeding cultivars that can maximize the use of photosynthetic capacity is recommended. For example, if the environmental conditions are conducive for increased photosynthesis and biomass, then an effectively selected cultivar should have sufficient capacity to use the additional photosynthate for formation of grain (Long et al., 2006). Certain cultivars fail to efficiently transform photosynthate into grain formation. Thus, beyond biomass, other factors such as high soil fertility, adequate water supply, and efficient cultivars need to combine to contribute to yield improvement (Li et al., 2023).

2.5.2 Phosphorus Tissue Concentration

According to Wieczorek et al. (2022), P concentration in plants ranges from 0.05 to 0.5% of plant dry weight regardless of the plant's growing conditions or specie. In 2022, P tissue concentration in flax was found to be higher ($P<0.05$) in the conventional treatment compared with the ORG M (Figure 2.2). Flax in the ORG NM was not significantly different than the other two treatments. All these treatments were considered P adequate since their P concentration percentage was equal and/or above the 0.20% sufficiency level (Han et al., 2022). In 2023, all the treatments attained P adequate concentration levels (above 0.20%). Flax in the CONV treatment presented the highest P concentration (0.46%) and although not significantly different, the ORG

NM treatment had more P concentration (0.41%) than the ORG M (0.37%). These results were contradictory to other similar studies. In their study, Komiyama et al. (2014) observed that livestock manure can build soil fertility and increase plant nutrient uptake of N, P, and K. Lu et al. (2020) made similar observations that soils treated with animal manure annually for a long-term period, 16 years, presented higher P concentration and P pools that were 1.2–3.8 and 0.8–1.9 Mg P ha^{-1}, respectively, greater than in the soils that did not receive any manure. However, as observed in my study, flax P tissue concentration did not positively respond to the addition of manure in the organic plots; the manure had little to no effect on the P tissue concentration levels of flax as the ORG NM flax presented a similar P tissue concentration to the ORG M. Although the reason for the contradictory results is not clear, the differences in P application rate was seen as a potential explanation; the P application rate in my study was at 'P replacement' level but the other studies added more P, hence its higher availability.

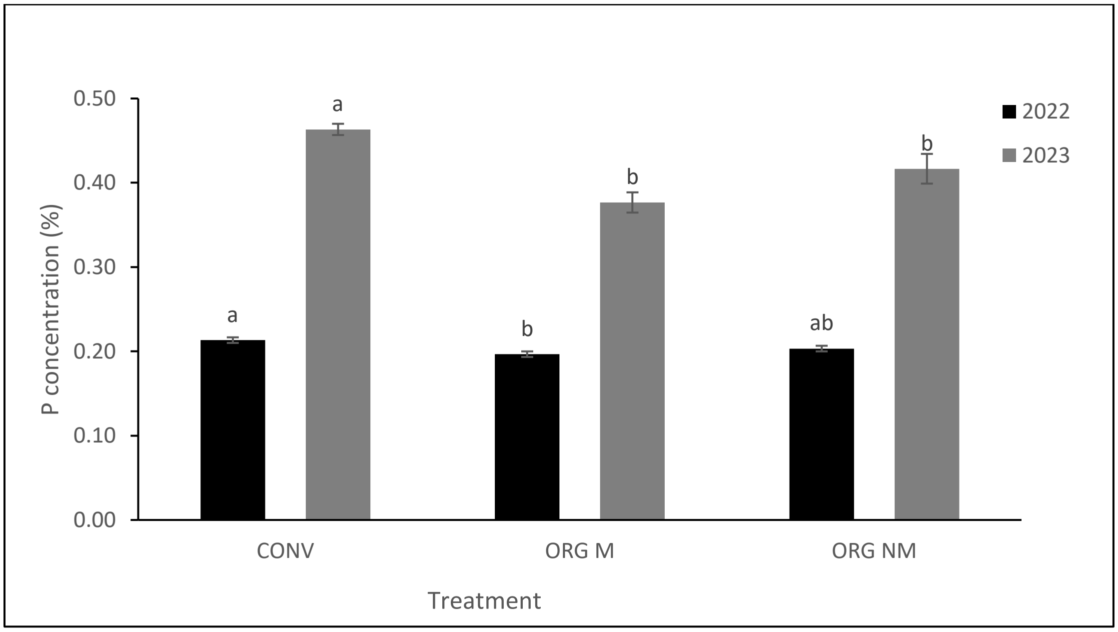

Figure 2.2. Shoot P concentration in flax at flowering stage from the conventional (CONV), organic manured (ORG M), and organic non-manured (ORG NM) treatments during the 2022 and 2023 planting seasons at the Glenlea long-term rotational field study. Samples taken in the month of July for both years. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the P<0.05 level, according to the Duncan's Multiple Range test (DMRT). Significance compared per year.

It was interesting to observe that the level of flax P tissue concentration was generally higher in 2023 compared to 2022 despite 2023 (321.7 mm total precipitation) being drier than 2022 (685.2 mm total precipitation). These results contradict Peuke and Rennenberg (2004) who observed a decrease in P tissue concentration when beech (*Fagus sylvatica*) seedlings were subjected to drought conditions; moisture unavailability limited phosphate mobility within the soil. However, the effect of drought on P tissue concentration of plants can be discussed from various points of view, including water scarcity, soil pH, and biomass reduction. Zhang et al., (2020) observed that abiotic factors such as drought affects soil phosphorus bioavailability by lowering soil pH which favors the solubilization and release of P held in calcium phosphate. This may be one possible explanation for more P available to the plants under the drier conditions in 2023 compared with 2022.

Another possible explanation for the rise of flax P tissue concentration in drier conditions was the reduction of biomass. There was an observation about flax response to soil phosphorus supply in regard to P tissue concentrations and aboveground biomass, especially within the organic system**.** As observed in 2023, lower flax biomass had a contrasting effect on its tissue phosphorus concentration, it doubled, rising from an average of 0.20% in 2022 to 0.42% in 2023. According to Jarrell & Beverly (1981), increased biomass production leads to a dilution of phosphorus in the tissue, referred to as the ‘dilution effect’. The ’dilution effect’ refers to changes in the concentration of an element in plant tissue caused by varying biotic (fungi, bacteria) and abiotic (temperature, light, soil moisture) factors. Hence, a reduction in soil moisture due to drought limited plant growth but enabled the nutrients to accumulate more in tissues of the stunted plants. Moeneclaey et al. (2022) also concluded that crop biomass can have a highly significant and negative effect on plant P concentration, after finding that plant species with high biomass had lower plant P concentrations.

2.5.3 Phosphorus Uptake

Flax P uptake was calculated by multiplying the biomass from each treatment by its corresponding P tissue concentration value. In 2022, the P uptake was observed to be higher with increasing soil P addition as seen in CONV and ORG M treatments (Figure 2.3). While ORG NM was significantly lower than the CONV treatment, the ORG M treatment was intermediate and not significantly different than the other treatments. Although the P uptake in main treatments was not significantly different in 2023, it was observed to be generally higher than in 2022 mainly due to the increased P tissue concentration levels favored by drought conditions and low biomass.

Although increase in biomass production was linked to increased soil fertility from P additions, nutrients from manure or fertilizer seemed to improve crop growth better under wet conditions (2022) than dry (2023). The response of crop growth to P addition has been observed by others studies to be improved when precipitation is adequate. Chtouki et al. (2022) evaluated the effect of soil moisture content on phosphorus uptake and growth of chickpea (*Cicer arietinum*) under three irrigation regimes (75% of field capacity (FC), 50% FC and 25% FC). The highest plant dry weight was obtained under the fertilized, 75% FC water conditions suggesting that the chickpea plants had access to adequate water and nutrition from an early growth phase which boosted their photosynthetic rate and ultimately, their growth. Similarly, P uptake was observed to be increasing and significantly higher under the fertilized, adequate water supply conditions (75% FC); chickpea plants presented the highest value of P uptake, 1.98 times higher than that of the unfertilized treatment with low soil moisture (25% FC). It appears that plants absorb nutrients more readily under optimum soil moisture conditions than under excessively wet or dry conditions. Excess water creates waterlogged conditions that eliminate oxygen from the soil; oxygen deficiency suffocates plant growth since plants need oxygen for cell division, growth, and nutrient uptake (Sharma and Kumar, 2023). Excessively dry conditions on the other hand, limit nutrient mobility especially P within the soil.

Therefore, it is possible to presume that the greater effect of manure on plant growth was observed under wetter conditions due to sufficient soil moisture that provided a conducive environment for well-functioning of microorganisms like AMF which facilitate more P uptake. AMF require water for their metabolic activities. Moreover, all growth processes including photosynthesis, nutrient absorption, and respiration need water (Sharma and Kumar, 2023).

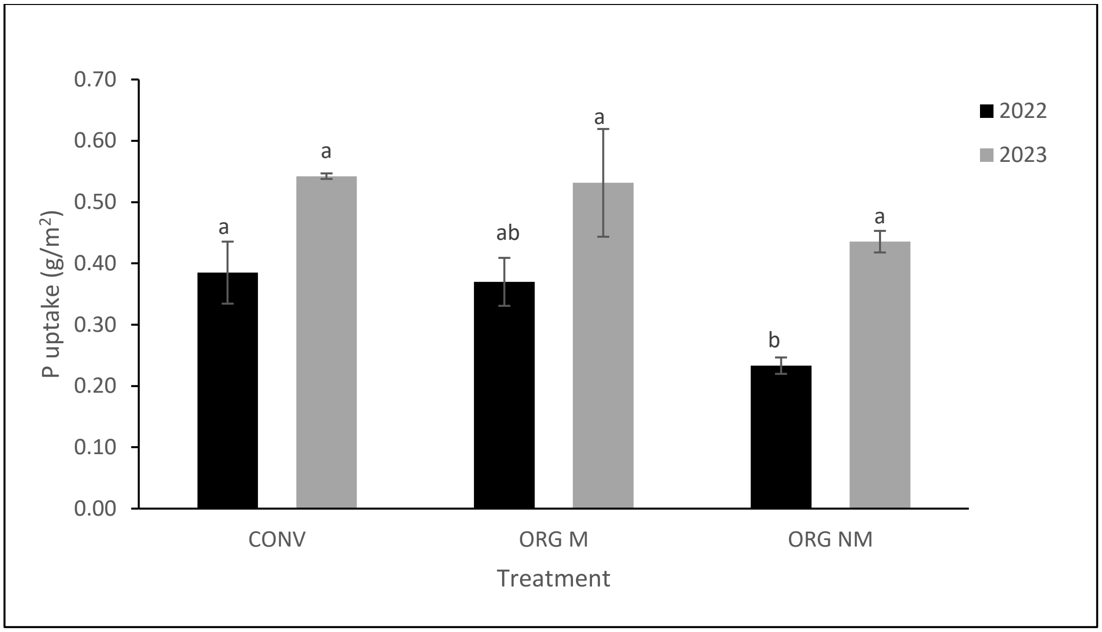

Figure 2.3. Shoot P uptake in flax at flowering stage from the conventional (CONV), organic manured (ORG M), and organic non-manured (ORG NM) treatments during the 2022 and 2023 planting seasons at the Glenlea long-term rotational field study. Samples taken in the month of July for both years. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the $P<0.05$ level, according to the Duncan's Multiple Range test (DMRT). Significance compared per year.

2.5.4 Arbuscular Mycorrhizal Fungi (AMF)

In 2022, the AMF (arbuscules) root count was the highest in the ORG NM treatment (40), followed by ORG M (37); both were significantly greater than the CONV treatment (17) (Figure 2.4). In 2023, the ORG NM treatment was significantly greater than both nutrient amended treatments and the ORG M was significantly greater than the CONV treatment. Overall AMF colonization was greater in 2023 compared with 2022; 2023 produced an average of 37% more AMF abundance than 2022. Shukla et al. (2013) conducted a study to determine the optimum soil moisture levels for AMF colonization during the early stages of development of various plant species; vigna mungo/black gram (*Phaseolus mungo* L.), wheat (*Triticum aestivum*), white siris (*Albizia procera*), and forest blue gum (*Eucalyptus tereticornis*). Three different levels of soil moisture were tested (FC, half FC, and double FC); results showed the highest AMF colonization at field capacity. The same study further states how both extreme drought and wetness can limit AMF due to inadequate growth of arbuscles structures from water stress and creation of anaerobic conditions from flooding. It was concluded that the 2023 drought level was favorable for the growth and extension of AMF although the exact soil water content was not identified in my study. Additionally, Duan et al. (2021) did an experiment to show the effect of AMF on wheat (*Triticum aestivum*) plants with and without AMF inoculation under two water regimes, 80% and 40% field capacity, (FC). It was observed that AMF inoculation significantly decreased shoot biomass, grain yield and water use efficiency in 80% FC, while it significantly increased these parameters in 40% FC, especially when plants were planted under high densities. AMF different performance under the two water regimes was seen as a sink-source relationship, where AMF inoculation enhanced the capabilities of sink acquisition and utilization under drought stress, while having no significant effect under the adequate watered conditions.

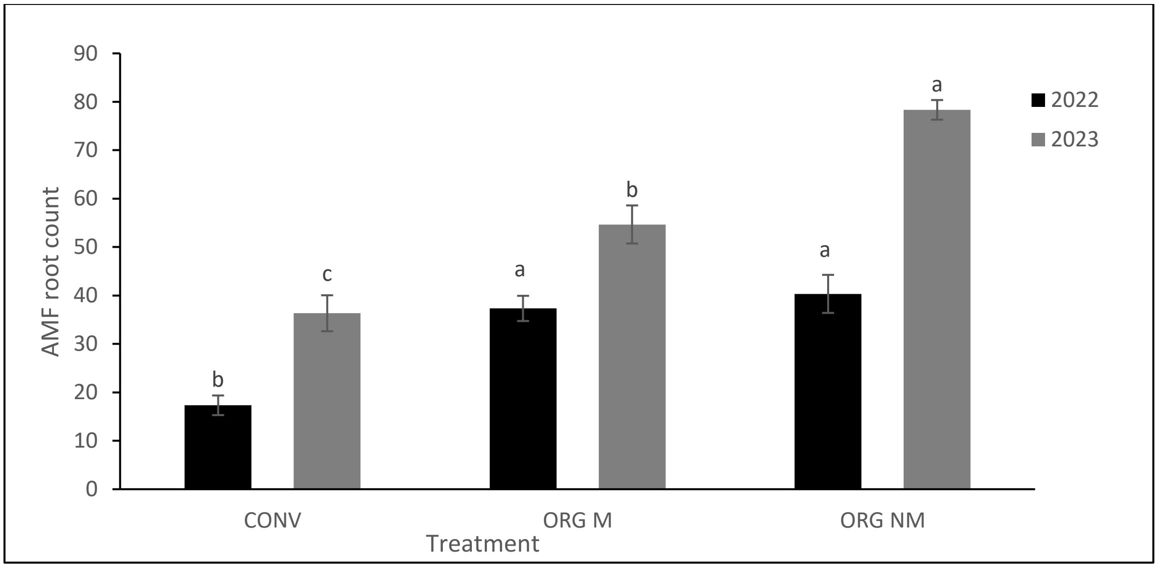

Figure 2.4. Root colonization by arbuscular mycorrhizal fungi in flax at flowering stage from the conventional (CONV), organic manured (ORG M), and organic non-manured (ORG NM) treatments during the 2022 and 2023 planting seasons at the Glenlea long-term rotational field study. Samples taken in the month of July for both years. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the $P<0.05$ level, according to the Duncan's Multiple Range test (DMRT). Significance compared per year.

Within the organic system, flax grown under ORG NM treatment had 30% more AMF abundance than ORG M. Based on previous studies, one explanation for higher AMF abundance in the non-manured system is lower soil P content (Dejana et al., 2022) . Soil in the ORG NM treatment had 6.8 ppm available P while the ORG M had 20.7 ppm Olsen P (Table 2.3). Therefore, lack of manure addition to the ORG NM plots appears to have created phosphorus deficient conditions that encouraged AMF root colonization to provide the crop with the needed assistance in scavenging for P. Low phosphate conditions allow the plant roots to form a symbiotic relationship with AMF for nutrient exchange (Tawaraya, 2022).

AMF abundance was found to be lower when P was added to the soil, as demonstrated in the ORG M, possibly because the addition of manure raised the soil P to a level a bit high (22.7 ppm in the present study) which limited AMF-plant interactions. This explanation is also supported by Shao et al. (2023) who illustrated how AMF colonization lowered with increasing P concentrations and vice versa. Similar observations took place when (Zhu et al., 2016a) investigated the effects of different fertilizations (NPK vs organic manure) on the variation of AMF community in the rhizosphere soil of a maize crop. The results showed that AMF diversity in the maize rhizosphere was significantly altered by different fertilization types; more AMF species were found in the organic manured maize rhizosphere than the synthetic fertilizer because the organic matter activated more AMF species to interact with other species, especially AMF species that were efficient in using organic matter as food to grow and reproduce.

Mycorrhizal abundance is considerably greater in organic systems than that in conventional ones (Fareed et al., 2024). Different agricultural management practices such as soil tillage, fertilization, crop rotation, and crop protection methods can influence AMF population and diversity (Mäder et al., 2000). For 15 years, Mäder et al. (2000) analyzed AMF root colonization in a long-term field trial comparing organic and conventional farming systems in a 7-year crop rotation where the results indicated that the percentage of root length colonized by AMF to be 30–60% higher in the organic than conventional system. AMF abundance was observed to decrease with the increasing fertilizer level in the conventional system. The different chemicals added to the conventional system ranging from pesticides, herbicides, insecticides, negatively affect the AMF-plant symbiotic relationship; the chemicals lower spore viability and hyphal growth thus limiting AMF development (Fareed et al., 2024). This observation is one of the reasons for the low AMF abundance encountered at Glenlea conventional treatment (Figure 2.4). During the planting season, the conventional plots receive chemical fertilizers in the form of urea and monoammonium phosphate to enrich the soil and although this has increased the N, P, K soil content (Table 2.3), it has negatively correlated with AMF abundance (Figure 2.4) because AMF is sensitive to high soil fertility levels. These plots are also sprayed with herbicides to control weeds, which can be harmful to the AMF network. Similarly, Braman et al. (2016b) evaluated the effect of organic versus conventional management systems on the soil microbial biomass phosphorus (MBP) at Glenlea. The MBP (mg MBP g^{-1}) was compared in organic manured, organic non-manured, and conventional treatments and the results showed that the microbial biomass phosphorus was more than double in the organic non-manured than in the conventional treatment while the organic manured was intermediate between the two. The organic system conditions were more favourable for microbial growth than the conventional one. Additionally, Fraser et al. (2015) examined the effect of management system, organic manured, organic non-manured, and conventional on soil P bioavailability and alkaline phosphatase activity after 20 years of field production. She observed significantly higher rates of phosphatase activity in the organic treatments compared to the conventional. These results concur with Krause et al. (2022) investigation on the effects of farming systems on microbial enzyme activities after 42 years of organic and conventional farming in the DOK trial; they noted that the organic management presented higher biological activities possibly due to higher presence of C from manure, which is food for microbes thus more microbial biomass recorded. The farm management system can influence soil AMF abundance and organic systems tend to provide more conducive environment for AMF developed compared to conventional.

Application of organic amendments rich in N can also enhance AMF growth by improving water-holding capacity and porosity of the soil which creates a more conducive environment for hosting AMF compared to chemical fertilizers (Aleklett and Wallander, 2012). N supply through organic amendments to P-deficient soils induces the development of AMF-plant symbiotic relationship because the added N forces the plant to seek more P supply for higher biomass production. The results of my study support this observation; flax amended with manure had a higher AMF colonization than where mineral fertilizer was used. Moreover, crop biomass from the organic manured treatment was not significantly greater than that from mineral fetrilizer (Figure 1). Addition of N can also introduce mycorrhiza-associated bacteria that helps facilitate AMF proliferation (Aleklett and Wallander, 2012). Therefore, N supply from organic amendments enhance more AMF biomass than inorganic N. However, adding N to P-rich soils shifts AMF-plant relationship from symbiotic to parasitic (Antunes et al., 2012); the presence of excess P reduces the need for AMF help thus the plant will invest its energy more in production than in AMF-plant partnership.

2.5.5 Effect of AMF on Flax Phosphorus Concentration

The correlation between AMF and P tissue concentration was analyzed to help answer the question: ‘Did AMF help the plant access P?’ Although an increase in AMF abundance was observed to have a positive effect on the flax P tissue concentration (Figure 2.5), the effect was as well closely connected to the soil moisture level. There was a large difference in P tissue concentration among the two years of the experiment, 2022 and 2023. In the wetter year (2022), flax P tissue concentration was seen to be lower than in the drier year (2023), although not significantly different. The wet conditions favored more P mobility within the soil which encouraged more biomass hence the P dilution effect. Under drier conditions of 2023, the effect of AMF on P was seen more; compared to 2022, P tissue concentration in 2023 was higher by 52%, slightly more than double. AMF increases plant growth and productivity by improving nutrient acquisition, such as phosphorus, water, and mineral uptake especially under abiotic stressors such as drought (Wahab et al., 2023). This explains why the effect of AMF abundance on flax P tissue concentration in the current study was more during the drier year. In their review Wahab et al. (2023) observed that AMF supported plant growth and nutrient absorption especially under dry conditions and raised plant P concentration by 33.0%. In another study, Qi et al. (2022) compared P concentration between AMF-inoculated and non-inoculated Canada goldenrod (*Solidago canadensis*) and noted that the AMF inoculation increased P concentration by 107% compared to the non-inoculated treatment. An increase in AMF promotes phosphate uptake in plants.

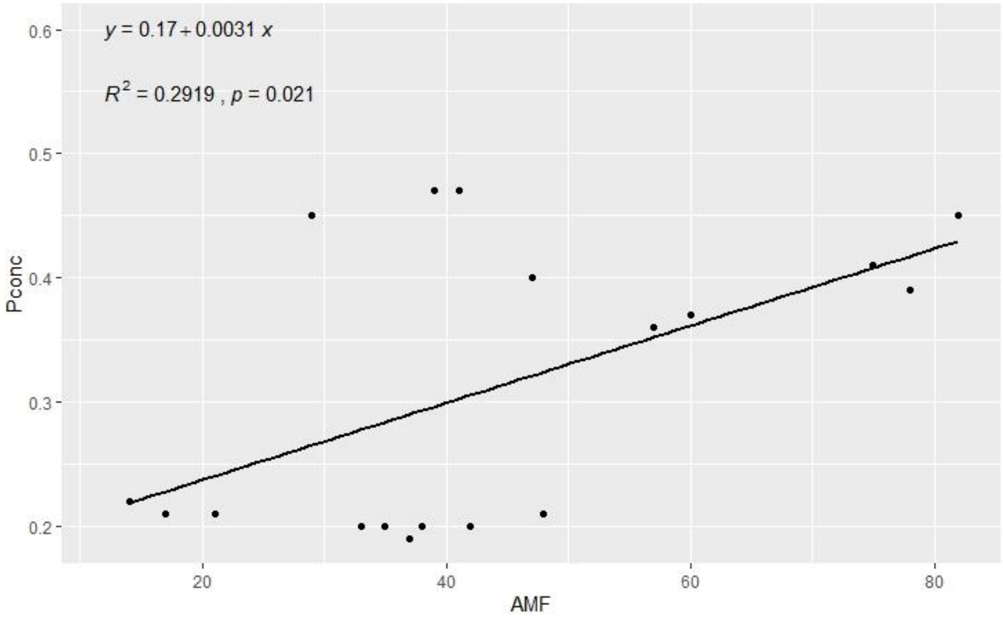

Figure 2.5. Effect of AMF on flax P tissue concentration. Equation of line of best fit is: y = 0.17+0.0031x (R^2= 0.2919). Combined data from 2022 & 2023 planting seasons.

2.6 Conclusion

The benefits of manure addition on flax growth were observed in a wet year compared with a drier year, providing evidence that soil moisture is required to facilitate P movement within the soil. Under wet soil conditions, ORG M treatment produced 33% more biomass than the ORG NM. Manure did not have a significant effect on flax P tissue concentration under both dry and wet soil conditions; adding manure did not improve flax P sufficiency as the ORG NM treatment presented 8% more P tissue concentration than the ORG M. AMF abundance was not significantly different in organic manured and non-manured treatment under wet soil conditions. However, under dry soil conditions, AMF colonization was 22% higher in non-manured than manured. A decreased abundance of AMF was observed when P fertilizer was added to the soil especially under wet conditions. However, flax amended with manure had higher AMF colonization than MAP-amended treatment. AMF play an important role in the nutrient absorption of flax, especially P. The relationship between AMF and P tissue concentration was positive; an increase in AMF led to a slight increase in flax P tissue concentration. For crops that are highly dependent on colonization of AMF for phosphorus uptake such as flax, the colonization of AMF increases the amount of phosphorus absorbed compensating for a less developed root system.

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3. Soybean Response to Organic Amendments in a Low Phosphorus Soil

3.1 Abstract

Limited sources of organic amendments for the supply of phosphorus limit the growth and production of soybean (*Glycine max* L.), especially in low input Canadian systems. Alternative organic sources of P need to be tested to give farmers more options. This study investigated various organic soil amendments as alternative sources of P under greenhouse conditions. Soybean was planted in P-depleted soil (3ppm Olsen-P) amended with five organic nutrients: composted beef cattle manure (CM), anaerobic digestate (AD), black soldier fly frass (FR), rabbit manure droppings (RM), ground rabbit manure (GRM) and compared to Mono-Ammonium Phosphate (MAP) and a control (CONT) (no amendments). Pots were filled with 2400 g of dry soil each and the amendments were all added at a rate equivalent to 20 kg P ha^{-1}. The amendments applied per pot were 13.1 g CM, 1.5 g AD, 4.6 g FR, 13 g RM, 0.18 g MAP, and 13 g GRM. Soybean was sampled for above-ground biomass, P tissue concentration, plant P uptake, and arbuscular mycorrhizal fungi (AMF) root colonization at the initial phase of flowering, 42 days after planting. GRM produced significantly higher ($P<0.05$) biomass, 34% more than RM, 35% more than CONT, and 19% more biomass than MAP. Similarly, GRM also presented the highest P tissue concentration, 18% higher than RM, 23% higher than CONT, and 9% more than MAP. P tissue concentration was observed as GRM > FR > MAP > AD = CM = RM= CONT. Soybean roots in FR treatment had the most AMF abundance while those in MAP produced the least count. The various organic nutrients influenced soybean growth differently based on the parameters measured. Rabbit manure performed better when ground than natural pellet form. An increase in AMF colonization improved plant P tissue concentration.

3.2 Introduction

The Library of Parliament, (2020) defines organic agriculture as a system of food production that seeks alternative sources for fertilization and disease-protection instead of reliance on synthetic fertilizers, pesticides, and biotechnology, a natural way of farming. Organic agriculture in Canada and around the world is experiencing steady growth due to: (1) growing consumer awareness and demand for food that is safe and free of any chemical residues that may threaten their well-being; and (2) higher food prices for certified organic products in the market that attract growers to grow organic food for increased income (Yang et al., 2023).

Soybean (*Glycine max* L.) is an important legume grown for its protein, oil, and micronutrients for both human and animal consumption (Mo et al., 2022; Yang et al., 2020). Comprised of approximately 40% protein and 20% oil content in its seeds on a dry mass basis (Li et al., 2021), soybean has gained prominence in Canada, particularly Southern Ontario, where its cultivation was initially confined until the mid-1970s due to the region's favorable warmer climate. However, propelled by advancements in plant breeding, the introduction of early maturing varieties capable of thriving in diverse climates has expanded soybean cultivation across Canada (Qian et al., 2022). Currently, soybeans are grown on more than 2.4 million hectares (5.9 million acres) of Canadian farmland and presently, Ontario, Manitoba, and Quebec produce most of Canada’s soybeans (Soy Canada, 2022).

Organic soybean farming remains limited on Canadian prairie farms due to several challenges encountered with its production. Lack of organic tested cultivars that can perform efficiently under organic conditions is a huge hindrance to adoption of organic soybean farming (Carkner, 2016). Majority of the cultivars present in the market were bred for conventional conditions and may not yield as expected under organic conditions. High weed pressure especially in the early plant development phases of soybean reduces its growth, quality and final yield (Van Acker et al., 1993). As a solution, organic farmers are advised to use narrow row spacing for better weed competition (Podolsky, 2014). Additionally, soybeans require slightly acidic soil, (pH ranging from 6 to 7) that facilitates the nutrient bioavailability to plants (Toomer et al., 2023). When grown in the Canadian Prairies, soybean is commonly included in crop rotations either as a monocrop or intercrop for its advantageous nitrogen-fixation ability. This natural nitrogen fixation not only benefits soybean growth but also provides residual nitrogen for succeeding nonleguminous crops like corn (*Zea mays),* wheat (*Triticum aestivum*), oats (*Avena sativa),* barley (*Hordeum vulgare*), and canola (*Brassica napus*) (BASF, 2020). One of the most popular combinations is the corn-soybean rotation because of corn's high nitrogen demand which can be eased through soybean's N fixation (Shea et al., 2020).

When it comes to soybean fertilization, nitrogen addition is often not a priority due to the natural nitrogen fixation of the bacterium *Bradyrhizobium japonicum* (Shea et al., 2020). However, phosphorus (P) deficiency is a common problem that limits soybean growth and yield and as Mo et al. (2022) reported, low P availability (< 5ppm Olsen P) could lead to crop yield reductions of 30–40%. P shortages arise from few approved P fertilizer resources for organic systems, increasing cost of P sources like manure, possible depletion of P sources that are mined (phosphate and rock) (Aziz et al., 2013;Mo et al., 2022). Additionally, limited soil moisture especially in the western prairies of Canada contributes towards P deficiency. Reduced soil moisture during some dry growing seasons on the prairies limits phosphorus mobility within the soil, thereby lowering crop uptake and subsequently, its yield (Qian et al., 2022; Yang et al., 2020). Phosphorus is also the least mobile nutrient under most soil conditions due to fixation by microbial activities and cations such as Al^{3+}, Fe^{2+}, Ca^{2+} in soils (Mo et al., 2022).

There are various mitigation strategies that can be implemented to solve the P-deficient issue in organic soybean farming. One of them is breeding P-efficient soybean varieties that can efficiently utilize native P and added P in the soil (Wang et al., 2010). Another strategy is the one discussed in the present study; application of organic soil amendments rich in organic matter. Organic amendments help rebuild the soil structure and increase moisture retention, creating a conducive environment for soybean growing (Li et al., 2021). When applied, organic amendments can boost soybean yields similarly or even higher than mineral fertilizers. For example, Bowden et al. (2010) observed that the application of organic amendments increased soybean yields by 21% and seed protein content by 4-9% above the control and inorganic fertilizer treatments. The same study further states that the hormones present in the humic substances of organic matter help in crop-drought alleviation.

The soybean plant itself has its own morphological strategies in adaptation to P starvation, such as optimizing root growth to scavenge for nutrients and formation of symbiotic association with beneficial soil microorganisms such as AMF (Jiang et al., 2021). AMF colonize plant roots to improve nutrient uptake for the host plant by mobilizing soil insoluble nutrients, especially phosphorus (Kuila and Ghosh, 2022). Compared to the plant's root system, AMF have the ability to reach and transport more P from deeper soil depths thus their much-needed assistance in moving immobile minerals like P (Kirk et al., 2011). While soybean roots host AMF to aid in P uptake, the roots also form a symbiotic relationship with rhizobium bacteria to form nodules for biological nitrogen fixation. There is a relationship between soybean nodulation and its P uptake because P deficiency limits nodule growth and development; P starvation reduces the number and size of nodules thus suppressing N fixation (Mo et al., 2022). It is therefore important that legumes such as soybean absorb adequate P.

The main objective of this study was to assess different organic soil amendments as alternative sources of P to soybean grown in P-deficient soil under greenhouse conditions. Other objectives included: (1) evaluating a novel manure source, rabbit manure, and compare its performance when in natural pellet form versus ground form and (2) testing if AMF colonization affects the ability of soybeans to uptake P. We hypothesized that soybean will produce more biomass and P tissue concentration when planted in organic soil amendments than in the mineral fertilizer. A second hypothesis stated that any increase in AMF would result in greater soybean growth and P uptake.

3.3 Materials and Methods

3.3.1 Nutrient Sources

Four organic nutrients: Black soldier fly frass, anaerobic digestate of food waste, composted beef cattle manure, and rabbit manure (in its natural pellet form and ground form), were investigated as potential sources of phosphorus for soybean grown in P-depleted organic soils. The organic nutrients were compared to the mineral fertilizer, Mono Ammonium Phosphate (MAP) and a control (no amendments). The rabbit manure was sourced from a small farm in Alberta while the other organic amendments and MAP were provided by the Plant Science department, University of Manitoba. All the manures were sent to Agvise Laboratories, North Dakota, USA for N, P, K analysis as shown in Table 3.1.

Table 3.1. NPK analysis of frass, anerobic digestate, composted beef cattle and rabbit manure

	N (G/KG^{-1})	P (G/KG^{-1})	K (G/KG^{-1})
Frass	32	8.7	8.7
Anaerobic digestate	38	28	40
Composted beef cattle manure	7.1	2.3	5.0
Rabbit manure droppings	13	0.15	0.25

3.3.2 Treatments and Experimental Setup

The greenhouse study was conducted at the University of Manitoba using soybean. The experiment used P-deficient soil (3 ppm Olsen extractable P) from an organic plot with a history of ten-year alfalfa production with no additional soil amendments in Libau, Manitoba. Libau's soil type is Orthic Dark Gray Chernozem. Soil for use in this study was collected from the plot were taken from five random points at 0–15 cm depth and analyzed at the Agvise laboratories with results of the analysis shown in Table 3.2. The coarse soil was then dried and sieved (approximately 5 mm) to obtain a uniform texture for easy mixing of the nutrients.

Table 3.2. Soil analysis results from the experimental areas (A-Trial plot) in Libau for 2022 growing season. Soil analyzed was top-soil, 0-15 cm

Nutrient	Value
Nitrate	22 kg/ha
Phosphorus (Olsen P)	3 ppm
Potassium	222 ppm
Boron	1.5 ppm
Zinc	0.58 ppm
Iron	20.7 ppm
Manganese	2.3 ppm
Copper	0.9 ppm
Magnesium	1032 ppm
Calcium	6016 ppm
Sodium	16 ppm

Organic matter	4.7%
Carbonate (CCE)	8.4%
pH	8.2

The experiment was divided into two "runs" that were conducted separately. "Run 1" was carried out from June to November 2022 and had six treatments, replicated four times. The treatments were T_1: Control (CONT), T_2: Cattle Compost (CM), T_3: Anaerobic Digestate (AD), T_4: Frass (FR), T_5: Rabbit Manure Droppings (RM), and T_6: Mono Ammonium Phosphate (MAP). Each of the 15 cm-diameter pots used was filled with 2400 g of dry soil. The amendments added to the potted soil were all applied at a rate equivalent to 20 kg P ha^{-1} and were mixed with the top 5 cm layer of the soil to facilitate the plant roots' early access to the nutrients. Nutrient amendments per pot were 13.1 g beef cattle compost, 1.5 g anaerobic digestate, 4.6 g frass, 13 g rabbit droppings, and 0.18 g MAP. "Run 2" was conducted from April to August 2023 and had the same treatments as "Run 1" plus the addition of one more; T_7 Ground Rabbit Manure (GRM). The rabbit droppings were dried, enclosed in a piece of cloth, and manually ground into powder-form using a hammer. Each pot received 13 g of GRM.

In both "runs", an organic-bred soybean variety, OAC Strive, provided by the Department of Plant Agriculture (Soybean Breeding & Genetics), from the University of Guelph, was planted. Pots were seeded with four soybean seeds per pot at an approximate depth of 1.5 - 2 cm. Seven-days post-emergence thinning was done reducing the number to two soybeans per pot. To ensure proper watering, the plants were watered manually based on the soil's field capacity; this was determined by calculating the difference between the weight of the dry soil (2400 g) and fully wet soil (3393 g). Approximately 65% of field capacity water was made available during the vegetative phases and 70% at flowering of the soybean.

3.3.3 Sampling and Data Collection

Soybeans were sampled for shoot biomass, P tissue concentration, plant P uptake, and AMF abundance. These measurements were taken during the initial flowering phase of soybeans and the evaluation of biomass, P tissue concentration, P uptake, and AMF followed the same procedures mentioned earlier in chapter 2 under 'Materials & Methods'.

3.3.4 Data Analysis

Data were analyzed using the statistical analysis software program, R and were checked for normal distribution using the Shapiro-Wilk test. The experiment was implemented twice (categorized as “Run 1” and “Run 2”) and was laid out in a completely randomized design. “Run 1” was carried out in 2022 and had 6 treatments with 4 replicates while “Run 2” was done in 2023 and had 7 treatments with 4 replicates. The treatments were considered as fixed effects while the replicates were the random effects. Difference among the parameters (dry matter biomass, P tissue concentration, P uptake, and AMF colonization) were tested using Analysis of Variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT), where means were considered statistically significant at $P<0.05$. Additionally, linear regression analysis was conducted to define the relationship between AMF colonization and plant P tissue concentration.

3.4 Results and Discussion

3.4.1 Biomass

A comparison of soybean biomass grown in different soil amendments under greenhouse conditions is presented in (Figure 3.1). In run 1, CM, AD, and RM produced soybean biomass similar to the control (unfertilized plants) and only MAP and FR had greater and significantly higher biomass. The highest soybean biomass was observed in plants treated with MAP (23.90 g) while among the organic soil amendments, frass produced the highest biomass (22.48 g). In “Run 2”, all treatments except GRM had similar biomass, for instance, CM, AD, and FR were similar to each other; CONT, CM, and RM were similar, and FR and MAP. When introduced in “Run 2”, GRM produced higher biomass (31.0 g) than all the other treatments; GRM produced 35% and 19% more biomass than RM and MAP respectively.

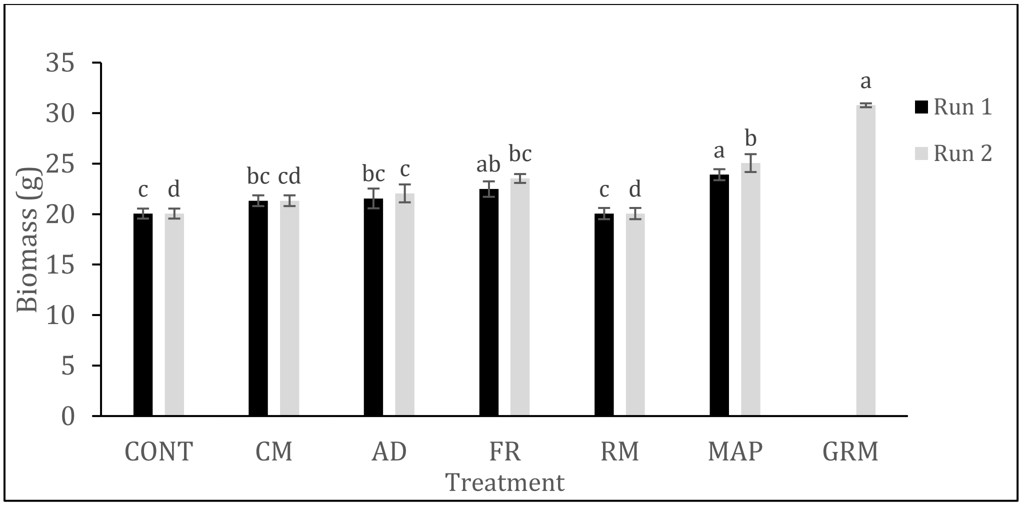

Figure 3.1. Soybean biomass at flowering stage. Plants were grown using different soil amendments under greenhouse conditions during Runs 1 and 2 of the experiment in Winnipeg, MB. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the $P<0.05$ level, according to the Duncan's Multiple Range test (DMRT). Significance compared per run.

MAP produced higher biomass than all the organic amendments except GRM. Higher plant growth in MAP-fertilized soil could be attributed to faster mineralization and access to nutrients in MAP than in the organic amendments (Titirmare et al., 2023). Compared to organic amendments, inorganic fertilizers are known to give fast response because they have a high nutrient content. The high nutrient content also makes them economical to farmers since they only require small quantities of application to achieve desired results. Furthermore, nutrients in inorganic fertilizers are available in a water-soluble form thus allowing for rapid absorption by plants (Titirmare et al., 2023). This observation is supported by Gogo et al., (2020) who did a study to compare the effects of superabsorbent polymer (SAP), rabbit manure, and control (no amendments) on soil moisture, growth and yield of eggplant (*Solanum melongena*). The results indicated that the highest shoot dry weight was produced from eggplant grown in SAP (305.6 g) > rabbit manure (298.7 g) > control (281.0 g).

Soybeans planted in frass produced competitive soybean growth and even had similar shoot dry weight to MAP. This is because nutrients from insect frass are easily assimilated by the plant roots. According to Poveda, (2021), insect frass experiences rapid mineralization with nutrients in a readily available form that boosts crop biomass as well as tissue nutrient content of crops like barley (*Hordeum vulgare*). This early release of nutrients to a short-season crop like soybean boosts the morphological growth of the plant. Similarly, Klammsteiner et al. (2020) observed the same in an experiment that compared the growth of perennial ryegrass (*Lolium perenne*) between frass and a mineral fertilizer (NH4NO$_{3);}$ there were no significant differences in the growth of perennial ryegrass between the treatments, indicating that frass serves as a rapidly acting fertilizer comparable to NH4NO$_3$.

Soybeans grown in GRM performed the best in terms of growth compared with other sources of organic nutrients and MAP fertilizer. Based on its competitive performance against the MAP fertilizer, GRM showed immense potential as an organic soil amendment. Potentially, rabbit manure could act as an MAP replacement as a source of P as it seems to have nutrients available to the plant roots similarly to a fertilizer. El-Mogy et al. (2020) noted no significant difference in the growth of lettuce (*Lactuca sativa)* planted in treatments of mineral fertilizer, chicken, and rabbit manure implying that the rabbit manure performed just as well as the fertilizer. Generally, better crop growth in rabbit manure has been linked to the manure's high N content that favors development of shoot growth. Rabbit manure is known to be one of the highest in N (2.40%) compared to others, beef cattle (0.73%), dairy (0.57%), and chicken (1.00%) (Kuepper, 2003). Similarly El-Mogy et al. (2020), noted that application of rabbit manure improved soil organic nitrogen (SON) that promotes shoot growth. In their study carried out to evaluate the effect of different organic and inorganic fertilizers on SON content of lettuce, plots supplied with rabbit manure produced the highest values of SON (1.5%) followed by chicken manure (1.2%), farmyard compost (1.1%), and lastly, mineral fertilizers (0.8%).

As observed in the current study, the performance of rabbit manure was influenced by its form. There was statistical difference between the plant dry weight of rabbit droppings in their natural form (RM) and ground form (GRM). Although some manures have to be pelletized to reduce storage, handling, and transportation costs (Key et al., 2023), rabbit manure comes naturally pelletized. However, the natural pellets might work against its speed of nutrient release to the plants especially under short-term growing conditions. Better crop response to GRM than RM could be attributed to the difference in size particles and nutrient availability of the two manures. Unlike the RM, GRM could easily and evenly be mixed with the soil and the even distribution allowed easier plant roots access to the nutrients. Although animal manure like rabbit can be directly applied as organic manure to plants, it is most effective when composted for faster crop response (Rodelio & Honeylet, 2021). Furthermore, the dry and hardened droppings needed more time to breakdown beyond the 42 days that the experiment lasted.

3.4.2 Phosphorus Tissue Concentration

The effects of different soil amendments on the P tissue concentration of soybean are presented in Figure 3.2. Under "Run 1" of the experiment, the decreasing P tissue concentration level was MAP > FR > CM > RM > AD > CONT. The CM, AD, FR, and MAP were not significantly different than each other but were all significantly different than the CONT and RM. Soybean grown in the CONT treatment had slightly lower P tissue concentration (0.19%), less than the P sufficiency threshold, 0.2% (line drawn across the graph). In "Run 2", GRM treatment presented the highest plant P tissue concentration and was significantly greater (*$P<0.05$*) than FR, which came second. MAP treatment was similar to FR. Both CONT, CM, and RM were not significantly different than each other but were significantly higher than AD (Figure 4.2).

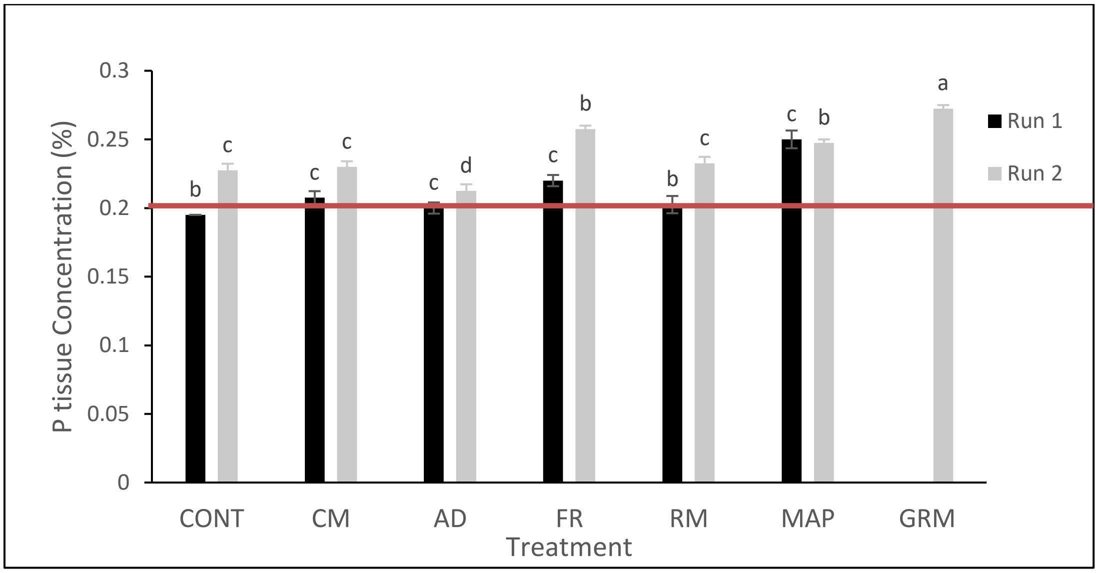

Figure 3.2. Soybean's shoot P concentration at flowering stage. Plants were grown in different soil amendments under greenhouse conditions in Runs 1 and 2 of the experiment, Winnipeg, MB. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the P<0.05 level, according to the Duncan's Multiple Range test (DMRT). Significance compared per run. The horizontal red line represents the P sufficiency threshold, 0.2%.

In “Run 2” GRM emerged as the highest organic nutrient in P tissue concentration and was significantly greater than the FR, the second highest. Plants grown in rabbit manure have been associated with higher plant nutrient uptake; (Kuepper, 2003) stated in his findings that rabbit manure poses as one of the top performing manures due to its high content of N, P, and K. Rabbit manure contains a higher % of phosphoric acid (1.40%) compared to other sources of animal manure, dairy (0.23%), swine (0.34%), chicken (0.80%), and beef steer (0.48%). Another study compared the influence of composted farmyard wastes, rabbit and chicken manure with mineral fertilizers on the soil chemical properties of lettuce. The highest value of soil P content was observed after the application of mineral fertilizer (100.02 ± 2.8 mg/kg) and rabbit manure (95.5 ± 2.1 mg/kg) (El-Mogy et al., 2020). Rabbit manure can supply P to plants almost similarly to mineral fertilizers. In the present study, GRM was not only higher but also significantly different than the mineral fertilizer, MAP. The high P tissue concentration in FR could possibly be due to frass quick mineralization of nutrients that are readily available for plant use (Poveda, (2021). Nutrients from insect frass are easily assimilated by the plant roots.

It was surprising to observe that the level of soybean P tissue concentration in anerobic digestate was not higher than the beef cattle compost. According to Nicksy and Entz, (2021), the solid fractions of anaerobic digestate tend to contain a greater concentration of phosphorus than their liquid counterparts. Additionally, Tampio et al. (2015) did research on characterization and usability of digestates from food waste and concluded that solid fractions from anaerobic digestion were highly suitable as fertilizers for leguminous crops because of their low nitrogen but higher potassium and phosphorus levels. It was therefore considered probable that soybean grown in anerobic digestate would be among the organic treatments with higher P tissue concentration but this was not observed. No obvious explanation for the poor performance of AD is available.

3.4.3 Phosphorus Uptake

The effects of different soil amendments on the P uptake of soybean are presented in Figure 3.3. The P uptake was calculated by multiplying the biomass from each treatment by its corresponding P tissue concentration value. Under “Run 1” of the experiment, the P uptake in the CONT, CM, AD and RM was similar but significantly lower than FR and MAP. The highest P uptake among the organic nutrients was observed in the FR treatment though it was significantly lower than MAP treatment. Similarly, in “Run 2” the CONT, CM, AD and RM were similar and significantly lower than MAP and FR, which were not significantly different than each other. GRM recorded the highest P uptake and was significantly greater than all the other treatments. GRM presented higher biomass and P tissue concentration compared with the other organic amendments hence the increased general P uptake.

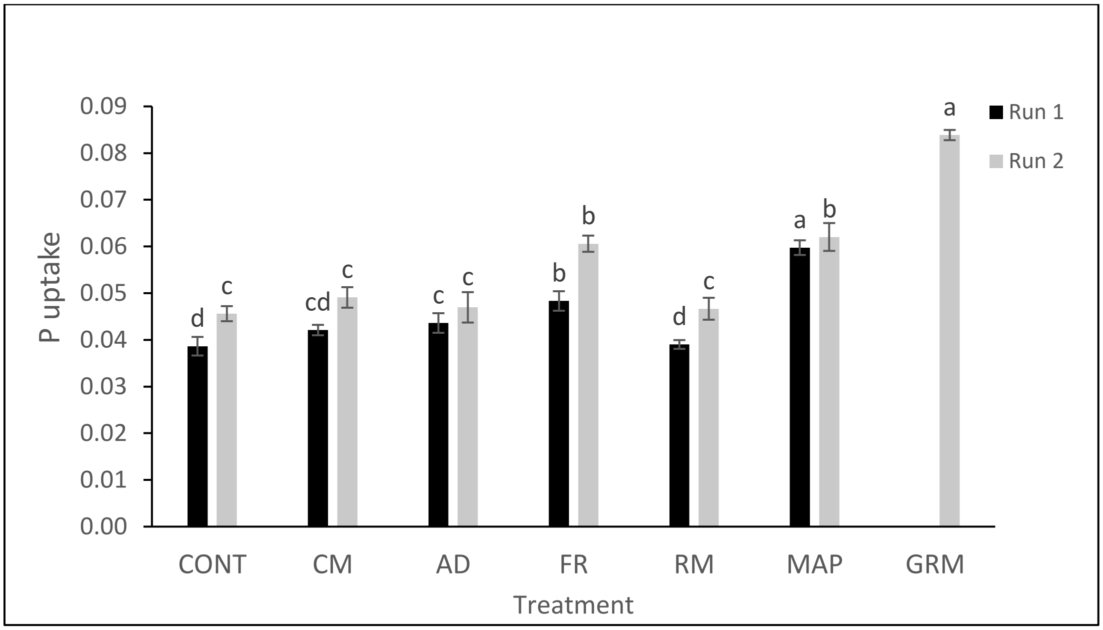

Figure 3.3. Soybean's shoot P uptake at flowering stage. Plants were grown in different soil amendments under greenhouse conditions in Runs 1 and 2 of the experiment, Winnipeg, MB. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the P<0.05 level, according to the Duncan's Multiple Range test (DMRT). Significance compared per run.

The P uptake results from most of the organic amendments were surprising. For example, the CONT, CM, AD and RM were not significantly greater in P uptake than the control. This observation was contrary to a study by (Tiwari et al., 2019) in which soybean uptake was higher in most of the soil-amended treatments than in the control (no amendments). In their study, they investigated the impact of inorganic fertilizers and farmyard manure application on nutrient uptake of soybean and the results indicated maximum uptake of P (8.35 kg ha^{-1}) in 100 % NPK + 5 t farmyard ha^{-1} treatment and the lowest value of P (1.84 kg ha^{-1}) in the control plot. In the current study, the uptake of P did not increase with the addition of the mentioned organic soil amendments. It was earlier assumed that the addition of P through the amendments would result in higher P availability in the soil for its subsequent uptake and use by plants. The underperformance by cattle manure (CM) could be due to immobilization of soil nutrients caused by high lignin content found in the manure which results from the feed eaten by the animal (Adekiya et al., 2020). Cattle feed, especially forages, contain high lignin and cellulose material that can slow down decomposition and release of nutrients, stunt plant growth thus lowering biomass which subsequently lower total P uptake. Unground RM (rabbit dropping pellets) presented low P uptake probably because the dry pellets appear in different sizes and shapes and do not easily mix evenly with the soil compared with ground rabbit manure.

Soybean P uptake in BSF frass was higher than in the control (non-amended) treatment; a result similar to a study by (Nicksy et al., 2021) in which the Italian ryegrass (*Lolium multiflorum*) grown under BSF frass showed increased and significantly higher P uptake compared with the non-fertilized control. In "Run 2" of the current experiment, P uptake between BSF frass and MAP showed no significant difference, a possible indication that the nutrient availability in BSF frass could be comparable to a mineral fertilizer. To support this observation, Choi et al. (2009) compared and found similar growth rate of Chinese cabbage (*Brassica rapa* L.) with BSF frass and a commercial fertilizer. Similarly, Nicksy and Entz, (2021b) and Nicksy et al. (2021) found a similar P uptake between BSF frass and MAP in a greenhouse pot study that compared struvite from municipal wastewater, BSF frass, anaerobic digestate, compost, to MAP and a control as sources of P for the growth of Italian ryegrass. The similar P uptake in soybean planted in BSF frass and MAP in my study supports the earlier mentioned similar studies. Additionally, Nicksy et al., (2021) also observed that BSF frass facilitated higher P uptake than anaerobic digestate although both amendments increased ryegrass P uptake compared with the control, P uptake rose by 70% with digestate and 130% with frass. Similarly in the current study, FR had 9.9% and 22% more P uptake in "Run 1" and "Run 2" respectively compared to AD. Thus, BSF frass could be a potential substitute to mineral fertilizers in agricultural fields.

3.4.4 Arbuscular Mycorrhizal Fungi (AMF)

A comparison of AMF abundance in soybean roots among different soil amendments is shown in Figure 3.4. In "Run 1" of the experiment, CONT, CM, AD, FR, and MAP did not present significantly different AMF abundance and only the RM was significantly greater than the control. In "Run 2" FR had higher AMF abundance, GRM was intermediate and similar to FR but not significantly different than other amendments except MAP.

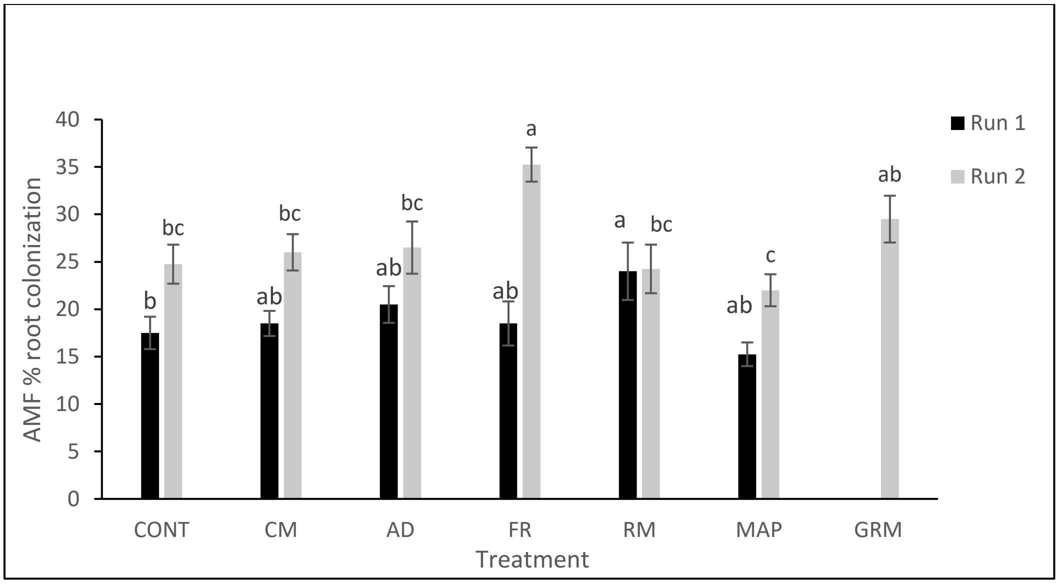

Figure 3.4. Root colonization by arbuscular mycorrhizal fungi in soybean grown in different soil amendments under greenhouse conditions in Runs 1 and 2 of the experiment, Winnipeg, MB. Values are mean and error bars represent standard errors. Means labelled with the same letter were not significantly different at the P<0.05 level, according to the Duncan's Multiple Range test (DMRT). Significance compared per run.

In "Run 2" all the organic amendments had either similar or greater AMF abundance than the MAP. This observation was also made by El-Mogy et al.(2020) in their study where indigenous soil mycorrhizal fungi in lettuce significantly increased in both population and density with the addition of organic fertilizers (rabbit and chicken) against chemical fertilizers. The increase was associated with the amounts of organic carbon present in organic fertilizers that provides food and energy enhancing the microorganism biomass and activity. Similarly, Zhu et al. (2016) carried out an experiment to measure differences in AMF species in maize rhizosphere between treatments with and without organic amendment. They observed that there were more AMF species in the manured treatment than in the non-manured. Generally, AMF seems to thrive in soil amended with organic matter due to the efficiency of some AMF species in using C derived from organic matter as energy to grow and reproduce (Cheeke et al., 2013).

Soybean grown in FR had the highest AMF abundance in "run 2" but not "run1". Poveda, (2021) stated that in addition to nutrients, insect frass also supplies the soil with other compounds like sugar which is a source of energy to microorganisms such as the soil beneficial fungi. This could explain why the frass-fertilized roots had the highest AMF abundance. However, it remains unclear why AMF abundance was high only in “run 2” considering all factors were kept constant during the whole greenhouse experiment. Frass is also said to be rich in organic carbon which sustains and promotes the growth of microorganisms like AMF (Watson et al., 2021). Moreover, the high moisture content combined with warm temperature of insect bred frass favor microbial growth (Klammsteiner et al., 2020).

The low AMF abundance in MAP is supported by previous studies. For example, in a meta-analysis of 162 field experiments with three treatments of organic fertilization, chemical-only fertilization and no fertilization, Jiang et al., (2021) found that the organic fertilization increased AMF colonization by 60% and AMF richness by 12% than the chemical-only fertilization. However, some studies disagree with the generalization that organic fields always present higher AMF abundance than conventional. Kim (2017) assessed if management systems (organic vs conventional) cause a difference in AMF colonization in strawberry (Fragaria × ananassa) roots; the results showed the organic fields had an AMF colonization mean of 51.25 while conventional fields had a mean of 55.32 which was not significantly different. According to Kim (2017), AMF abundance and composition is more influenced by the specific farming practices such as crop species, rotation, and tillage than by the general management system. In the current study, organic treatment, with or without manure addition, had higher AMF abundance than conventional. The organic system creates an AMF-conducive environment by eliminating chemical farm inputs that threaten AMF development as well as providing C as food for the fungi.

3.4.5 Effect of AMF on P Tissue Concentration

The relationship between AMF and P tissue concentration was evaluated to test whether AMF colonization affected the ability of soybeans to uptake P. As shown in Figure 3.5, AMF had a positive relationship with P tissue concentration hence an increase in AMF abundance could be associated with an increase in P tissue concentration.

Various studies have assessed the relationship between AMF and plant nutrient uptake. The symbiotic associations formed between AMF and plant roots in AMF-host species not only enhance plant nutrient uptake but also stress tolerance and overall growth and development (Wahab et al., 2023). It is expected that plants with more AMF colonization achieve a higher P uptake because AMF root colonization expands the absorptive surface area of the root system allowing for more nutrient absorption (Qi et al., 2022). In areas where the indigenous AMF population is limited, more AMF can be introduced through inoculation. Qi et al. (2022) measured P tissue concentration in Canada goldenrod (*Solidago canadensis)* by comparing an inoculated and a non-inoculated specie. They observed that the AMF inoculation increased P concentration by 107% compared to the non-inoculated treatment. AMF inoculation significantly promoted phosphate uptake. Similarly, Lu et al. (2023b) also evaluated the influence of AMF on nutrient uptake of wild maize (*Zea mays*) by comparing P uptake between the AMF inoculated and non-inoculated maize after 8 weeks of growth. The results showed significant difference as AMF inoculation increased the leaf P concentrations of wild maize by 33.86% compared with non-inoculated wild type. The presence and addition of AMF in the maize rhizosphere soil improved its P concentration. An increase in AMF colonization leads to an increase in P tissue concentration.

AMF can improve nutrient uptake for their host plant. Nguyen et al. (2019) did a study to measure AMF colonization of barrel medic (*Medicago truncatula*) plant that was grown in different soil P levels, low and high (exact values not specified). They observed that AMF colonization raised the plant's P tissue concentration at low soil P, AMF colonization was highest (mean 75.8%) when P was limiting and lowest (mean 8.7%) when P increased. However, in my study, while AMF had a positive relationship with P tissue concentration, not all low P treatments presented higher colonization. For instance, CONT, CM, AD, FR, and MAP did not present significantly different AMF abundance despite control having low P and the manured treatments having high P. This could be that the manures added did not make a difference in soil P levels within the experiment's timeline.

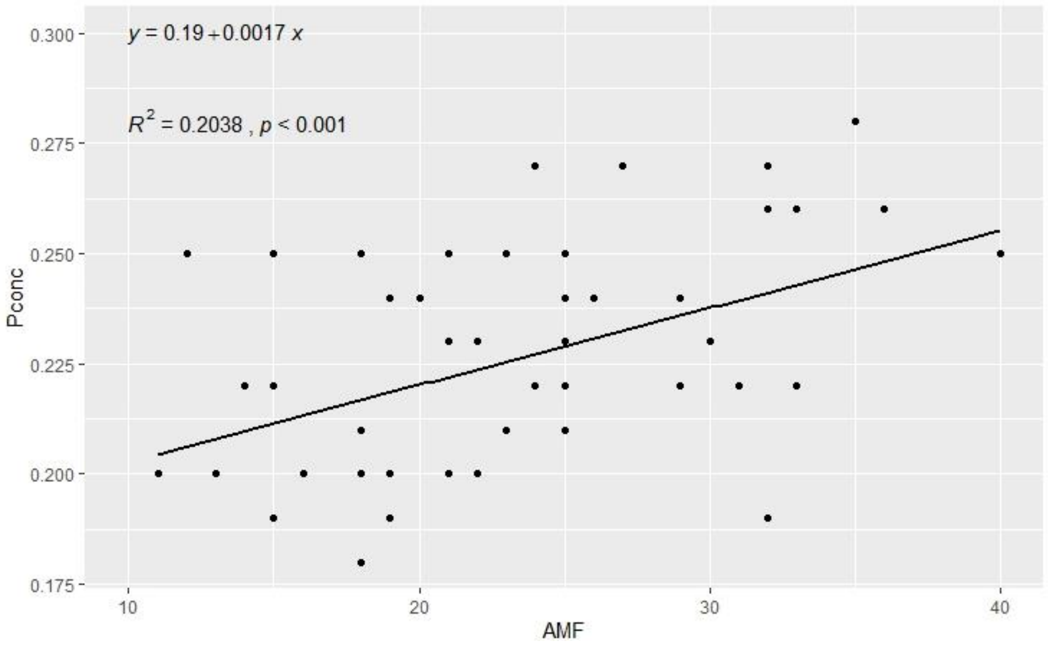

Figure 3.5. Effect of AMF on soybean P concentration. Equation of line of best fit is: y = 0.19+0.0017x (R^2 = 0.2038). Data shown is from combined "Run 1" and "Run 2" experiments.

3.5 Conclusion

There is not enough livestock manure in the world to cater for every organic farmer, and thus there is need for education, exposure, and experimentation with alternative manure sources from insects, food waste, and novel livestock like rabbits. Rabbit manure is naturally pelleted and upon drying, it can be used directly without composting; as it does not cause a burning effect on crops. However, ground rabbit manure is easier to mix evenly with the soil compared to the natural dried pellets that are not uniformized in shape and size. Affordable anaerobic digester material, for example, the Costa Rican tubular PVC membrane bags, culturally called "sausage digesters" should be advertised to more farmers for low-cost infrastructural that may encourage them to recycle more food waste for a circular agricultural economy. In this study, Anaerobic Digestate (AD) did not perform as expected especially with the soybean P concentration; despite being a high P manure, AD did not increase soybean's P concentration and the reason is unknown. Similarly, cattle manure's impact in the greenhouse study was not beneficial especially when compared to the emerging alternatives such as frass and rabbit manure.

4. GENERAL DISCUSSION AND CONCLUSIONS

The main objective of this study was to assess different organic soil amendments as alternative sources of P for soybean and flax and their comparison with fertilizer. Percentage root colonization of AMF was measured to evaluate if AMF colonization is increased in low phosphorus organic systems leading to an increase of phosphorus absorption in plant tissue. Plant biomass was taken to determine the influence of farm management system on plant growth rate. P tissue concentration was analyzed to assess the impact of manure addition on plant P uptake. The different parameters were measured for soybean and flax under greenhouse and field conditions respectively. The questions tested were: (1) Did manure addition improve plant performance in terms of biomass, P uptake, and AMF colonization; and (2) Did AMF help plants access P.

Canadian organic farmers heavily rely on livestock manure and phosphate rock as sources of P. Unfortunately, P mined from the phosphate rock is unreliable because it not only faces extinction but also poses environmental harm through its mining (Cordell and White, 2011). Although livestock manure is renewable, its provision is restricted due to the limited number of livestock on most Canadian farms as well as the rising manure prices and transportation costs (Aziz et al., 2013). As a result, alternative organic sources of P are needed hence this study investigated novel manures including rabbit and black soldier fly frass as possible options.

4.1 Rabbit Manure

I am of the opinion that more farmers need to adopt commercial rabbit farming and not just for the financial benefit but largely for its abundant manure production that can alleviate the manure deficit challenge that most organic farmers currently face. I did this study with a keen interest on the performance of rabbit manure alongside other organic soil amendments. This study accomplished to showcase the potential of rabbit manure to increase crop growth especially plant biomass and P tissue concentration. Additionally, there is need to promote the production of small livestock such as rabbits and insects for increased production of manure with less damage to the environment. In agriculture, livestock production is responsible for 18 % of greenhouse gases (GHGs) emitted annually due to animal respiration, land requirements, growth of feed crops, and methane production due to decomposition of manure. Currently, most meat products are obtained from the large mammals (swine and cattle) which are the major producers of GHGs due to their large necessities of land, water and feed (Goodland and Anhang, 2009).

An increase in the production of small livestock like rabbits could be a potential solution to mitigating GHGs as small livestock require less land, less food, have a higher proliferation rate, greater food conversion ratio, and have faster growth rates (Maharaj et al., 2006). Due to their small body size, rabbit production occupies less space in comparison to other livestock such as cattle and can be domesticated in cages thus occupying very little space making them ideal for minimal land sizes. Rabbits have a long lifespan of 8 to 12 years, during which a single rabbit can produce approximately 28.8 kg of manure, and high levels of nitrogen and phosphorus in comparison to other animal manure from sheep, goats, pigs, chickens, cows, and horses and the manure is also termed as ‘cold manure’ because when dried, it does not burn crops when added directly to the soil without decomposition (Kuepper 2003). From this study’s results, it was also evident that the performance of rabbit manure could be influenced by its form i.e. crop response varies depending on the size of the manure particles. As observed in this study, ground rabbit manure mixes uniformly with the soil to facilitate quick nutrient access to short-seasoned crops like soybean. If used in its natural pellet form, the droppings should be applied at least 2 months prior to planting to give them enough time to decompose and release nutrients to plants upon planting.

Rabbit manure comes naturally pelleted unlike other manures used in this study such as cattle and anaerobic digestate that have to be manually pelleted. Pelleting is a mechanical process that compacts the solid fraction of manure at high temperature and pressure to convert it into a uniformized and dry finished product referred to as pellets (Sharara et al., 2018). Uniformity of particle sizes is important to ensure that it is evenly distributed during land application (Sharara et al., 2018). Certain factors, like the moisture content and particle size of the raw material or manure influence the quality of the produced pellets. In their research, Pampuro et al. (2017) observed that a moisture content higher than 75–80% creates an unsuitable environment for the pelleting and also lowers the pellet durability, the optimal moisture content for pelleting varies between 20% and 40% while and ≤ 10% moisture is considered too low.

According to Pampuro et al. (2017), the pelleting process comes in as a solution to increase the bulk density of most solid manure fractions to improve their transportation and application on farms; pelleting is known to raise bulk density from an initial value of <200 kg m^{-3} to a final value >1000 kg m^{-3}. The drying stage during the pelleting process lowers the weight of the manure hence easier for transportation and storage (Szogi et al., 2015) . Pelleting enables to process the manure with a consistent nutrient content to match specific crop nutrient requirements; this can reduce surplus nutrients particularly phosphorus, whose excess causes eutrophication (Sampat et al., 2019). In addition, pelleting manure minimizes odor and flies through the drying process. On the other hand, the pelleting process is expensive and the cost varies depending on the type of equipment and binder used. “In addition, manure pelleting involves both pretreatment and post-treatment steps; the pretreatment process includes solid-liquid separation and drying while the post-treatment can include screening and milling” (Sharara et al., 2018). Pelleting capital and labor intensity are not feasible for small or medium farms. The capital cost for such system is considered economically worthy for very large farms (around 15,000 dairy cows) or a cooperative of several farms (WaterWorld, 2001). Another limitation with pelleting is the high-energy consumption, particularly when relying on natural gas for manure drying which can increase the carbon footprint of animal production (Szogi et al., 2015). An alternative could be low-carbon drying systems, such as the sun or greenhouse drying.

4.2 Insect Frass

Another valuable ‘small livestock’ is insects. Insect farming has captured the interest of organic agriculture due to its dual role in transforming organic food waste into natural plant fertilizer and providing protein for both human and livestock diets (Gärttling & Schulz, 2022). Insects such as the Black Soldier Fly (BSF) can be bred and fed on a specific diet to produce a valuable end-product, frass (insect poop), which is dried, ground and used as manure. Application of organic soil amendments such as frass stimulates the abundance and activities of beneficial indigenous microbes in the soil (Barragán-Fonseca et al., 2022). Moreover, amendment-mediated stimulation of indigenous microorganisms is preferred compared to addition of commercial microbial inoculants because the indigenous soil-borne microbes are well adapted to local soil conditions. The efficiency of commercial inoculants is not guaranteed because the introduced inoculant may be incompatible with the plant species or the new inoculant and local AMF communities might mismatch (Li, 2016b). Rearing BSF for insect frass protects the environment by diverting food waste from landfills while at the same time, producing a high-quality manure.

4.3 General Conclusions

When the organic amendments were applied separately as in the case of the greenhouse study, ground rabbit manure (GRM) and frass were observed to outperform the rest (anaerobic digestate, beef compost, rabbit manure pellets). Soybean planted in GRM had higher biomass and tissue P concentration than composted beef manure, anaerobic digestate and monoammonium phosphate but similar to insect frass. Additionally, GRM and frass treatments presented higher mycorrhizal colonization. In the case of the field study where the three manures (frass, beef compost, and anerobic digestate) were mixed to make a blend, the effect of the manure blend on flax was influenced by other factors such soil moisture. For example, flax biomass increased with the addition of manure but this observation was made only under wet soil conditions. Under dry conditions there was no significant difference between the biomass of the organic manured (ORG M) and organic non-manured) flax. Addition of manure did not improve flax P sufficiency; ORG NM treatment had more P tissue concentration than the ORG M. AMF abundance was not significantly different between the ORG M and ORG NM except under dry soil conditions, where AMF colonization was higher in ORG NM. Moreover, a decreased abundance of AMF was observed when P fertilizer was added to the soil especially under wet conditions. Generally, the organic treatments had higher AMF colonization than the conventional-amended treatment.

4.4 Future Research

For future research, the root biomass of flax should also be measured and compared with its shoot biomass to determine the relationship between flax’s aboveground and belowground growth. In the current study, I observed that the drought conditions experienced in 2023 reduced flax shoot biomass and that was similar to the findings of other studies. However, drought influence on biomass allocation differs between the shoot and root and it would be interesting to test if that is the case with flax as well. In a meta-analysis that investigated the impact of drought stress on biomass allocation, Eziz et al. (2017) found out that under drought situations, plants invest more in developing a root system than the shoot because a deeper root network will facilitate better water uptake from belowground while less aboveground growth will minimize water loss through transpiration. Additionally, plants respond to water scarcity by decreasing their stomatal activity to minimize water loss which consequently lowers photosynthesis and general shoot growth (Bista et al., 2020). Another study by Seleiman et al. (2021) confirmed that under drought conditions, there is a low shoot to root ratio; water deficiency causes plant morphological changes such as reduced leaf area, plant height, and shoot length hence lower shoot biomass. Although I observed flax shoot biomass to decrease with decreasing rainfall, its AMF abundance increased with decreasing rainfall, hence the interest to investigate its root biomass especially under drier soil conditions. The root biomass will help answer the questions: (1) Does flax increase its root biomass under drier soil conditions to increase nutrient and water uptake? (2) Does an increase in AMF abundance influence flax root biomass? And (3) Does the drought influence on biomass allocation differs between the shoot and root in flax?

In addition to rabbit manure, I recommend rabbit urine as another source of nutrients that requires more research to enlighten organic farmers on its application, benefits, and potential in food production. An adult rabbit produces approximately 100-300 ml of urine per day (Indabo and Abubakar, 2020). When diluted in the ratio 1-part urine and 3-parts water, rabbit urine is one of the most nutritious foliar fertilizers that can be used to increase crop production due to its various advantages: (1) It contains high content of nitrogen and also provides phosphorous and potassium to the crops ; (2) It is an ecofriendly foliar fertilizer that can be absorbed by plants directly through the pores and also through the roots; (3) The urine produces ammonia which acts as a repellent to pests thus also termed as an organic insecticide (Kemunto et al., 2022; Said et al., 2018). According to Lebas et al. (1997), the application period for rabbit urine to crops starts one week after planting in the case of seedlings but two weeks after sowing in the case of seeds. The application is done two weeks later with seeds because a seed contains nutrients and does not need extra fertilizer immediately. Moreover, the plants need to develop leaves first before the start of foliar applications. Rabbit urine applications should cease at least three weeks before harvest as a precaution against any microbes that may have been present in the urine mixture; most microbes in urine have been found to die off in three weeks (Lebas et al., 1997). Rabbit urine is known to work best on leafy vegetables, cereals such as maize, millet, and sorghum, and also on tomatoes (Indabo and Abubakar, 2020; Kemunto et al., 2022). Rabbit urine has been used in African countries including Ethiopia, Kenya, Uganda, Tanzania, Malawi and Nigeria as a pesticide and fertilizer; the urine has been sprayed on crops like maize to kill aphids (Kemunto et al., 2022). It would be interesting to carry out a study on the use of rabbit urine as an organic insecticide to control aphids on canola grown in the Canadian Prairies.

Another future recommended research topic is, ‘How does natural pelleted and manual pelleted manure compare? Some of the questions that could be answered in the research include: (1) Would manually pelleted manure perform better based on its particle size uniformity and specific nutrient content? (2) Apart from time, what are the other limiting factors that influence the breakdown and nutrient release of pelleted manure? Animal species? Moisture content? Soil temperature? And (3) Should naturally pelleted manures like rabbit, alpaca, and goat be ground for faster assimilation by plant roots?

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